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(54) Title: METHOD OF CREATING A BIOSTATIC AGENT USING INTERPENETRATING NETWORK POLYMERS

(57) Abstract

Applicants' invention is a method for creating an interpenetrating network on the surface of devices and supplies that is biocompatible and antimicrobial. According to Applicants' invention, a polymerizable or monomeric quaternary ammonium salt in a solvent is exposed to a polymeric substrate. The quaternary salt in solvent is absorbed by the polymeric substrate and the quaternary salt is polymerized such that an interpenetrating network is formed with said polymeric substrate.

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METHOD OF CREATING A BIOSTATIC AGENT USING INTERPENETRATING NETWORK POLYMERS

BACKGROUND

Quaternary ammonium salts have the general formula of:

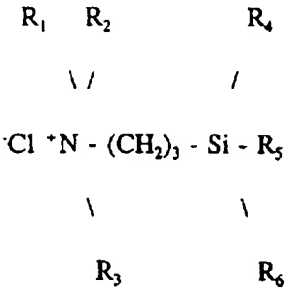
(1) $[(CH_3)_4 N]^+ X^-$

where X is a halogen such as iodine, chlorine or bromine. A variety of quaternary ammonium compounds are available and widely used as disinfectants and biocides and to treat items that may undesirably support microbial growth. For example, quaternary ammonium salts are used to treat carpeting, walls, various commercial products such as sponges and fabrics, and even water. They are also used to rehabilitate "sick buildings," particularly after floods and water leaks, and reduce odors caused by mildew, fungus and bacterial growth in damp basement areas.

Most quaternary ammonium salts commercially available are generally pre-packaged in water or alcohol solutions of approximately 2-3% or less quaternary salt concentration. They are applied to substrates such as carpets, walls, floors, to kill the bacteria. The method of application often relies on delivering the quaternary ammonium salt in a fine spray. When treating fabrics, sponges, bedding, and similar products, the concentration of the quaternary ammonium salts generally can be much lower, e.g., less than 1%.

Despite knowledge of the common usage of quaternary ammonium salts for imparting antimicrobial properties, a method was not known for treating medical devices and supplies and other consumer products that was biocompatible.

Applicants' method uses quaternary ammonium salts of the general formula of:



(2)

wherein R_1 and R_2 are methyl ($-CH_3$) groups; R_3 is octadecyl ($CH_3(CH_2)_{17}-$); and R_4, R_5 and R_6 are methoxy ($-OCH_3$) groups. Applicants' method can be used to treat, either during or after manufacture, textile materials, particularly medical devices and supplies, such that such devices and supplies have long-lasting, non-leaching, biocidal properties on the surface and are not toxic to the host organism. The treatment involves converting the methoxy groups to OH groups through hydrolysis and then polymerizing through condensation of the OH groups to form siloxane bonds and water.

More specifically, because catheter infections are the leading cause of hospital or long-term care infections, numerous attempts have been made to create a catheter that is antimicrobial. Most antimicrobial catheters rely on the impregnation of antibiotics to achieve a catheter that is resistant to bacterial infection. Unfortunately, this use of antibiotics results in increased resistance to antibiotics, a significant problem for immuno-compromised patients. It also leads to the subsequent long-term inefficacy of such catheters.

Further, some antimicrobial catheters use a coating treatment to provide a vehicle for entrapping drugs onto the catheter surface but permit subsequent diffusion into the

biological environment. Many such treatments rely upon a polyurethane in a solvent to entrap antibiotic pharmaceutical agents.

Thus, despite numerous and concerted efforts, a cost-efficient method has not been devised to impart non-leaching, biocompatible, antimicrobial properties to surfaces. In particular, despite the long felt need for such method or device in the catheter industry, until Applicants' invention, no such method or device existed.

Interpenetrating polymer networks (IPNs) are well known in the art. They are prepared in a variety of ways and the technical literature is replete with the technology for the manufacture of such IPNs. The most common ways to create IPNs are (1) by blending two or more polymers in an internal mixer using temperature, mixing time and torque to obtain a blended or grafted IPN, and (2) by "swelling," i.e., expanding, a higher polymer with a monomer or a solution of a monomer and polymerizing the monomer to a polymer in situ.

In this latter case, when monomer (A) is polymerized to form a polymer (A) in a host or substrate polymer (B), such as silicone or polyurethane elastomer, a high degree of permanence can be established for polymer A. That is, polymer A can only be removed to a limited degree when the IPN is extracted by an organic solvent or water. Therefore, such an IPN has long term stability.

However, until now, IPNs of polymerized quaternary ammonium salt monomers have not been used to impregnate the surfaces of medical devices and supplies to impart antimicrobial properties to such devices and supplies. Applicants' technique accomplishes this in such a manner that does not compromise their biocompatibility.

It is an object of this invention to provide a method for creating an interpenetrating network on the surface of devices and supplies that is biocompatible and antimicrobial.

It is a further object of the invention to provide a method for creating a biocompatible and antimicrobial surface for consumer products.

It is an object of the invention to incorporate antimicrobial activity into devices that may be implanted in or used on living organisms.

It is a further object of the invention to provide an antimicrobial catheter that is not dependant on antibiotic drugs for antimicrobial activity.

It is an object of this invention to provide a process for creating a polymeric coating having antimicrobial properties that can be applied to various medical device and supply surfaces.

Other objects of the invention will be obvious upon reading the following specification and claims.

FIELD OF INVENTION

This invention relates to a novel way to treat surfaces such that they have a non-leaching antimicrobial property that is not dependant on antibiotic drugs. The method described herein may be used to prepare or treat biocompatible devices or other products and impart antimicrobial properties to surfaces through coatings containing the antimicrobial.

SUMMARY OF THE INVENTION

Applicants' method is a technique for impregnating a surface with quaternary salts that have antimicrobial characteristics and are polymerizable. Applicants' technique calls

for the creation of an IPN of the quaternary salt in or on the material to be treated. In one embodiment of Applicants' method, the quaternary salt is polymerized after it has penetrated the surface of the host polymer, i.e., the polymer on the surface of the device or product to be treated. The depth of the penetration of the quaternary salt in the host polymer is controlled by the period of time that the polymeric substrate is exposed to the solution containing the quaternary salt, and solvent power, i.e., how much of the solvent is adsorbed by the subject device or product during the exposure period. The solvent power is reflected by the weight gain of the subject device or product during the exposure period.

After the quaternary salt monomer has been absorbed by the host polymer, the quaternary salt is polymerized to form an interpenetrating network polymer (IPN). Such polymerization preferably is achieved by using 0.1 N NaOH, 0.1 N HCl, heat or a combination thereof. The presence of the interpenetrating polymer (i.e., the active quaternary ammonium group) has been substantiated by a dye test using bromophenol blue. The longevity or permanence of the quaternary ammonium group has been demonstrated by dye testing the treated material after repeatedly challenging the treated host substrate with multiple hot (e.g., 140°F) water rinses, aging treated samples with forced air or in a microwave oven, and subjecting the treated sample to repeated autoclave cycles (270°F for 30 minutes).

As the following non-limiting examples show, the IPNs of silicone and polyurethane rubber, including silicone and polyurethane rubber catheters, treated according to Applicants' method, have been shown to possess the ability to kill bacteria, fungi and molds.

In other embodiments of Applicants' invention, a non-leaching antimicrobial IPN is created within the cavities and pores of the material to be treated. This embodiment does not require that the material to be treated be swelled. Rather, the quaternary salt monomer/solvent are absorbed into the pores, the solvent is evaporated and the monomer is polymerized within and through the pores of the substrate. In this manner the polymerized quaternary salt is "anchored" to the substrate through a physical interaction or blending. The level of quaternary salt polymer should be less than about 5% by weight on the substrate to minimize the decrease of air flow through the polymer substrate.

Another embodiment of Applicants' invention provides for the creation and application of a polymeric coating that can be applied to a variety of non-polymeric surfaces.

PREFERRED EMBODIMENTS

Applicants' method uses the technology of swelling a host polymer with a solvent solution of quaternary ammonium salt. Preferably, the solvent is selected based on its ability to swell rapidly the host polymer the desirable amount without significantly disrupting the integrity of the underlying host substrate. Even more preferably, the appropriate and necessary amount of swelling of the host substrate, e.g., as reflected by an approximately 20 to 50 percent weight gain of the solvent and quaternary salt, occurs within 10 minutes or less after exposure to the solvent. Even more preferably, the boiling point of the solvents are relatively low to facilitate the removal, i.e., the evaporation, of the solvent from the substrate being treated. The following non-limiting examples reflect application of Applicants' invention.

Example One - Quaternary ammonium salt IPN polymer on thermoplastic polyurethane rubber catheters (TPU)

Applicants' method was applied to a commercially available polyurethane rubber catheter, i.e., the host polymer. A solvent solution of quaternary ammonium salt was employed. Specifically, commercially available quaternary ammonium salt products were used that provided for different concentrations of a quaternary salt in methanol solution. The selected solvent was used to prepare 1-5% solutions of quaternary ammonium salt in ethyl acetate. This solvent was chosen because of its ability to rapidly induce the swelling of the underlying substrate polymer. The solvent in this example caused a thermoplastic polyurethane rubber catheter to exhibit approximately 30% weight gain in approximately 5 minutes. Such swelling was measured by weight gain attributed to the solvent and quaternary ammonium salt when compared with an untreated device or product. The catheter hub swelled slightly less than the catheter tube as the following Table 1 shows:

Immersion Time in Ethyl Acetate 5% Quaternary Ammonium Salt, minutes	% wt. gain of TPU Catheter	% wt. gain Hub from Catheter
1	16.8	8.7
2	15.4	13.8
5	30.8	20.8
10	40.5	-

This disparity in weight gain between the hub and the other portions of the catheter tube may be caused by the hub being thicker in cross section than the catheter tube or the hub being made of a different thermoplastic polyurethane.

After swelling in ethyl acetate, the swollen catheter was immersed in 0.1 NaOH to accelerate the polymerization of the quaternary ammonium salt. The clear 0.1 N NaOH solution became slightly cloudy indicating that some of the monomeric quaternary ammonium salt was dissolved or leached from the surface of the catheter and polymerized in the 0.1 N NaOH solution. However, a significant amount of polymerized quaternary ammonium salt remained on the surface and penetrated the catheter wall to a slight degree.

Standard Test A - Bromophenol Blue Testing

Successful treatment of the catheter was verified by exposing the treated catheter surface to bromophenol blue which colors the substrate blue in the presence of monomeric or polymeric quaternary ammonium salt. Additionally, a treated catheter segment was subjected to a 5 x series of hot water rinses (140°F tap water 200:1 on a shaker for 3 minutes) followed by a test with bromophenol blue. This sample also turned blue indicating that the IPN retained its activity and was not easily extracted. If desired, deeper penetration of the catheter wall can be achieved by increasing the immersion time or using a more powerful solvent. However, more powerful solvents or longer exposure time in the solvents, could result in a longer drying time to reduce the retained solvent content to acceptable levels. Further, the length of the exposure time must be calibrated for non-crosslinked polymers to ensure that the integrity of the underlying product or device to be treated is not compromised.

Standard Test B - Bio Testing of TPU Catheters

Catheters treated as described above, were submitted for and subjected to biotesting, i.e., testing for efficacy in living organisms. In one experiment, staphylococcus epidermidis (ATCC 12228) was harvested from a secondary working culture and grown to a concentration of approximately $1x10^8$ CFU/ml. Ten colonies were incubated at 35-37°C for 4 hours in trypticase soy broth ("TSB"). The culture was diluted to 10 $1x10^5$ CFU/ml by serially diluting in sterile, room temperature phosphate buffered solution. Test and control groups were comprised each of fifteen 1.0cm segments sectioned from a commercially available catheter that was not coated with any known antimicrobial compound. Ten ml of inoculum was pipetted onto each test and control segment and air dried at room temperature for 35-40 minutes. Each segment was placed in a vial containing 3.0 ml of sterile, room temperature TSB. The vials were shaker incubated (110 rpm at 35-37°C). After 1.0 hour of incubation, five vials containing test segments and five vials containing control segments were removed. The segments were removed from each vial. Each vial was vortex mixed, on high speed, for two minutes. The TSB in each vial was sampled (1.0 ml) and serially diluted six times in sterile, room temperature phosphate buffered solution for drop counting. This process was repeated for test and control vials after 4 and 20 hours incubation.

The results are summarized in Table 2 as follows:

Table 2 - Summary of Results, CFU/ml

Dilution Level	Control Segments	Micro organism Concentration Incubation time 1 hour	4 hours	20 hours
1:10	C_1	TFTC	$2.0x10^3$	$6.3x10^8$
1:100	C_2	TFTC	$2.0x10^3$	$6.1x10^8$
1:1,000	C_3	TFTC	$1.5x10^3$	$1.0X10^9$
1:10,000	C_4	TFTC	TFTC	$6.9x10^8$
1:100,000	C_5	TFTC	$1.6x10^3$	$7.7x10^8$

Dilution Level	Treated Segments	Incubation Time 1 hour	4 hours	20 hours
1:10	T_1	TFTC	TFTC	TFTC
1:100	T_2	TFTC	TFTC	TFTC
1:1,000	T_3	TFTC	TFTC	TFTC
1:10,000	T_4	TFTC	TFTC	TFTC
1:100,000	T_5	TFTC	TFTC	TFTC

TFTC = Too few to count (<30 CFU/ml)

These results indicated that the treated segments produced an inhibitory effect on the growth of s. epidermidis (ATCC 12228). Two routes of inhibition are possible: (1) contact inhibition, beginning with the initial inoculation of the catheter segments (either occurring in a dry environment or occurring when the coating moistened while in the TSB); or (2) while submerged in the TSB, the coating's inhibitory agent leached from the segment surface and circulated freely within the TSB.

Standard Test B2 - Bio Testing with C. albicans

To confirm that the treated catheters are resistant to a variety of bacterial organisms, a series of tests were conducted using candida albicans, ATCC 10231. C. albicans was harvested from a secondary working culture and grown to a concentration of about 10^7 CFU/ml (20 colonies incubated in 5.0 ml TSB at 35-37°C for 4 hours and 100 rpm). The culture was diluted to $1x10^5$ CFU/ml by serially diluting in sterile, room temperature TSB. As described for the foregoing test with S. epidermidis. Test and control groups were established each having fifteen 1.0 cm segments that were sectioned from a commercially available catheter, where the test group was from a treated catheter and the control group was from an untreated catheter. Ten ml of inoculum was gently pipetted onto each test and control segment and air dried under laminar air flow for 35-40 minutes. Each segment was then placed into a sterile vial containing 3.0 ml of TSB. The vials were shaker incubated (100-110 rpm) at 35-37°C. After four hours of incubation, five vials containing test segments and five vials containing control segments were removed from incubation and the segments removed from the vials. The TSB in each vial was sampled (1.0 ml) and serially diluted four times in sterile, room temperature phosphate buffered solution ("PBS") for drop counting. The process was repeated for samples removed at 8.0 and 20.0 hours of incubation.

The data from these tests is summarized in Table 3 as follows:

Dilution Level	Control Segments	4 hours Incubation	8 hours Incubation	20 hours Incubation
1:10	C_1	TFTC	TFTC	$4.1x10^5$
1:100	C_2	TFTC	TFTC	$7.5x10^5$
1:1,000	C_3	TFTC	TFTC	$4.1x10^5$
1:10,000	C_4	TFTC	TFTC	$6.8X10^5$
1:100,000	C_5	TFTC	TFTC	$1.9x10^5$

Dilution Level	Treated Segments	4 hours Incubation	8 hours Incubation	20 hours Incubation
1:10	T_1	TFTC	TFTC	TFTC
1:100	T_2	TFTC	TFTC	TFTC
1:1,000	T_3	TFTC	TFTC	TFTC
1:10,000	T_4	TFTC	TFTC	TFTC
1:100,000	T_5	TFTC	TFTC	TFTC

TFTC = Too few to count (< 30 CFU/ml)

From these data it can be concluded that the treated catheter segment had an inhibitory effect against C. albicans when compared to an untreated control. The experimental results indicate that the material from treated catheters does not leach when in PBS. Thus, the inoculum is inhibited upon contact with the treated catheter surface, either during the inoculum drying period or while immersed in TSB.

Standard Test B3 - Bio Testing with S. aureus MR

Staphylococcus aureus (ATCC 33591) was harvested from a secondary working culture and grown to a concentration of $1x10^8$ CFU/ml. Ten colonies were incubated for five hours in TSB at 35-37° and 100 rpm. As described above, the culture was serially diluted to obtain a culture concentration of $1x10^5$ CFU/ml.

Fifteen test and fifteen control group catheter segments, each 1.0 cm, were sectioned from a commercially available catheter. Ten ml of the 10^5 inoculum was gently pipetted on each catheter segment and allowed to dry under a laminar air flow for 30-35 minutes. Each segment was placed in a sterile vial containing 3.0 ml of TSB. The vials were shaker incubated (100-110 rpm) at 35-37°C. After four hours of incubation, five vials containing test segments and five vials containing control segments were removed from the incubator. The segments were removed from the vials and the TSB was vortex mixed on high speed for 2 minutes. The TSB in each vial was sampled (1.0 ml) and serially diluted four times in sterile, room temperature PBS for drop counting. Samples were also taken after 4 and 20 hours of incubation, diluted six times and drop counted to determine organism concentration.

These data are summarized in the following Table 4:

Table 4 - Micro Organism Concentration

Dilution Level	Control Segments	Incubation 1 hour	4 hours	20 hours
1:10	C_1	TFTC	$7.7x10^4$	$9.5x10^7$
1:100	C_2	2333	$6.7x10^4$	$7.3x10^7$
1:1,000	C_3	2000	$8.1x10^4$	$6.3x10^7$
1:10,000	C_4	TFTC	$7.5x10^4$	$9.5x10^6$
1:100,000	C_5	TFTC	$6.8x10^4$	*

* contamination made counting S. aureus colonies impossible to read

Dilution Level	Treated Segments	1 hour	4 hours	20 hours
1:10	T_1	TFTC	TFTC	$8.8x10^5$
1:100	T_2	TFTC	TFTC	$3.8x10^4$
1:1,000	T_3	TFTC	TFTC	TFTC
1:10,000	T_4	TFTC	TFTC	$2.3x10^4$
1:100,000	T_5	TFTC	TFTC	$4.7x10^4$

TFTC = Too few to count (<30 CFU/ml)

These data reveal that Applicants' method significantly reduced, but did not completely inhibit, the growth of methicillin resistant S. aureus (MRSA). The untreated control segments showed no sign of inhibiting the growth of MRSA.

Standard Test C - Assay of Agent Leaching

One cm catheter segments, treated with Applicants' method, were placed in five vials with 30 ml of PBS each, and shaker incubated (100 rpm) at 35-37° for 20 hours ± 5 minutes. These were assayed for active ingredient with ultraviolet light ("UV") or infrared ("IR") from 200 to 1000 nm. Similarly, control catheter segments were prepared and evaluated using UV or IR. No difference between control and test spectra were observed.

In another test, five segments, each 0.5 cm long, of catheters treated with Applicants' method were vertically inserted into 20 ml ± 5 ml trypticase soy agar ("TSA") inoculated with 1-2 x 10^6 CFU/ml of s. epidermidis (ATCC 12228). The petri dish containing the agar (TSA) and segments was incubated at 35-37° for 24 hours ± 15 minutes in air. The area around each catheter segment was examined for reduction or inhibition of microbial growth visible in the size and/or density of colonies, i.e, the zone of inhibition ("ZOI"). The size of any area of inhibition was measured. Control samples also were established. The data obtained are summarized below in Table 5:

Assay Part	Group	Media	Organism	Segment Numbers 1	2	3	4	5	Avrg.
C	Test	TSA	S. epidermidis	0.0	0.0	0.0	0.0	0.0	0.0
D	Test	STSA	S. epidermidis	0.0	0.0	0.0	0.0	0.0	0.0
E	Control	TSA	S. epidermidis	0.0	0.0	0.0	0.0	0.0	0.0
F	Control	STSA	S. epidermidis	0.0	0.0	0.0	0.0	0.0	0.0
G	Test	TSA	C. albicans	0.0	0.0	0.0	0.0	0.0	0.0
H	Test	STSA	C. albicans	0.0	0.0	0.0	0.0	0.0	0.0
I	Control	TSA	C. albicans	0.0	0.0	0.0	0.0	0.0	0.0

J	Control	STSA	C. albicans	0.0 0.0 0.0 0.0 0.0 0.0

Wherein S. epidermidis is ATCC 12228; STSA is soft trypticase soy agar and C. albicans is ATCC 10231.

The ZOI screening test produced no visible reduction in density of colonial growth of either S. epidermidis or C. albicans after 24 hours of exposure.

The spectrometry and ZOI evidence indicates that substantial leaching of active compound from the treated catheters does not occur. Accordingly, Applicants' invention allows beneficial bacteria to exist in biological systems but does not permit the growth of bacteria on treated surfaces. Further, because the active compound does not leach, Applicants' method operates to permanently impart the antimicrobial characteristic to the treated surface.

Example Two - Quaternary ammonium salt IPN polymer on silicone catheters

In another set of experiments, Applicants evaluated various solvent mixes to determine the degree of swelling of commercially available silicone catheters. The purpose of these experiments was to identify solvent blends that would result in excess of 25-30% weight gain after a 5 minute immersion. Thirty percent or more weight gain has been deemed the weight gain reflecting optimization of adequate penetration of the solvent (and the quaternary ammonium salt dissolved in the solvent) into a silicone rubber matrix.

The swelling results are shown below in Table 6 using a commercially available silicone rubber catheter:

Solvent Mixture	Approximate Immersion Time, Min.	% Wt. Gain
75 methanol 25 THF	0.25	6.3
75 methanol 25 THF	5.0	6.3
50 methanol 50 THF	5.0	24.6
25 methanol 75 THF	5.0	52.3
0 methanol 100 THF	5.0	85.1

Applicants used 25 methanol/75 THF solution and approximately 52% weight gain for these experiments. Again, catheters were exposed to a 5% solution of quaternary ammonium salt in 25 methanol/75 THF followed by 5 minute exposure to 0.1 N NaOH, approximately 30 minutes to an hour air drying, followed by forced air drying.

Standard Test A - Bromophenol Blue Testing

The bromophenol blue test was used on the treated silicone catheter which indicated the presence of the impregnants by the surface of the treated catheter turning blue. As with the polyurethane catheter, the silicone catheter was given a 5x series of rinsings in 140°F hot water of 3 minutes at approximately 200:1 on a shaker with vigorous agitation. Retesting with bromophenol blue dye indicated that the polymerized quaternary ammonium salt was not extracted from the body of the catheter.

Standard Test B - Bio Testing of Silicone Catheter Segments

Samples of untreated silicone catheter (control) and a treated catheter were evaluated against S. epidermidis. The challenge organism, S. epidermidis, was harvested and standardized to 1 x 10^8 CFU/ml. The suspension was diluted to approximately 1 x 10^5 CFU/ml. Several one cm pieces of each type of catheter were inoculated with 0.01 ml of the 10^5 CFU/ml suspension to give a final inoculum of 1 x 10^3 CFU per piece. Each piece was allowed to dry in a sterile dish for approximately 10 minutes and then placed in a vial containing 3 mls of TSB. The vials were incubated at 32-35°C for two days and evaluated for growth. The treated catheters killed the challenge organism. By challenging the TSB from the vials showing no growth without the catheters, Applicants demonstrated that the treated catheters did not leach the antimicrobial agent.

Additional silicone catheter segments were tested for ZOI against S. epidermidis, S. aureus and C. albicans with no evidence of leaching. The results are shown below in Table 7:

Catheter Sample Identification	S. epidermidis	S. aureus	C. albicans
Silicone rubber control	0.0	0.0	0.0
Treated silicone rubber catheter	0.0	0.0	0.0

Although Applicants' experiments focused on the application of Applicants' method to catheters, it is readily apparent to those skilled in the art, that other polymeric surfaces, particularly those present in medical devices, may be subjected to Applicants's method.

Standard Test C - In Vivo Bio Testing of Silicone Catheter Segments

In one experiment, a silicone catheter was prepared as described above using a 5% solution of quaternary ammonium salt in toluene solvent and followed by polymerization using 0.1 N NaOH as a catalyst and heat to remove residual solvent. This treated catheter was implanted in a rabbit to determine whether Applicants' method, when applied to a catheter, inhibits bacterial growth following active challenge with an organism at the site of implant. The treated catheter was implanted subcutaneously and S. aureus in a volume of 50 μl was deposited at the site. A control catheter was implanted in another animal. After 15 days of implantation, the treated and untreated catheters were removed, streaked across an agar plate, incubated and the colonies were counted. The colonies generated by the untreated catheter were too numerous to count (greater than 100) while only seven colonies were generated by the treated catheter. The test protocol and test results reflect the effectiveness of treating catheters with a polymerized quaternary ammonium salt.

As the foregoing experiments demonstrate, Applicants' method can be used to create a catheter having non-leaching, antimicrobial properties. Imparting such a characteristic to a catheter that has leaching antimicrobial properties, e.g., one that has antibiotics impregnated therein, may result in a catheter that is able to address an existing systemic infection that may affect the catheter surface. Applicants' process does not preclude the addition of antibiotics as a coating surface. Thus antibiotics can be used in conjunction with a surface that has been treated according to Applicants' method.

Although Applicants' experiments focused on the application of Applicants' method to catheters, it is understood that other polymeric surfaces, particularly those present in medical devices and supplies, may be subjected to Applicants' method.

Example Three - IPN in porous substrates

Another embodiment of Applicants' method does not require the host polymer substrate be capable of being swelled in a solvent. In this embodiment, the quaternary salt monomer/solvent mixture is allowed to penetrate the pores or interstices of the host polymer or substrate, the solvent is evaporated and the quaternary ammonium salt monomer is polymerized in situ. Polymerization is accomplished by heat, 0.1 N NaOH, 0.1 N HCl or a combination thereof. This results in an IPN in which the quaternary salt polymer is entangled in the pores of the host polymer or substrate. Applicants have used their method in host polymers having pores of approximately 2 microns. The host substrate can be a polymer such as Teflon or a variety of plastic or sponge-like materials such as foams and includes natural products such as paper. Using this procedure the quaternary salt polymer/host polymer IPN is highly stable and exhibits permanence as evidenced by (1) resistance to 5X hot water rinses for three minutes at 140°F and (2) resistance to up to 10 autoclave cycles for 30 minutes at 270°F. In each case, the blue dye test demonstrate the presence of the quaternary ammonium salt polymer after exposure to the elevated temperature.

Example Four - IPN Coating

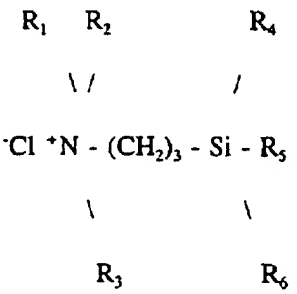
Further, it is apparent that Applicants' method may be used to create a polymeric IPN coating that can be applied to other solid substrates, including, but not limited to, substrates made of metal and plastic. For example, a polymerizable quaternary ammonium salt monomer at approximately 5% concentration, based on the resin solids, may be added to a commercially available coating system. The coating, with the

quaternary salt, may then be applied, e.g., by brushing or spraying, to the metallic surface to be coated. As the coating dries, the quaternary salt provided by Applicants method simultaneously polymerizes. Using this method Applicants successfully treated copper, aluminum, steel and stainless steel, but it is understood that other solid substrate surfaces, e.g., wood and plastic, can be treated. Blue dye testing verified the presence of the polymerized quaternary ammonium salt polymer in the coating system when the coating system was an epoxy paint.

WHAT IS CLAIMED IS:

1. A method of imparting antimicrobial properties to a polymeric substrate comprising: providing a polymerizable or monomeric quaternary ammonium salt in a solvent; contacting the polymeric substrate to said solvent containing said quaternary salt; permitting said quaternary salt to be absorbed by the polymeric substrate; and polymerizing said quaternary salt such that an interpenetrating network is formed with said polymeric substrate.

2. A method according to claim 1 wherein said quaternary salt has the general formula of:



3. A method according to claim 2 wherein R_1 and R_2 are methyl groups, R_3 is octadecyl, and R_4, R_5 and R_6 are methoxy groups.

4. A method according to claims 1, 2 or 3 wherein said polymerization of said quaternary ammonium salt is achieved by exposing it to 0.1 N NaOH, 0.1 N HCl, heat or a combination thereof.

5. A method according to claims 1, 2 or 3 wherein said solvents for said quaternary ammonium salt rapidly swell said polymeric substrate to a degree necessary to

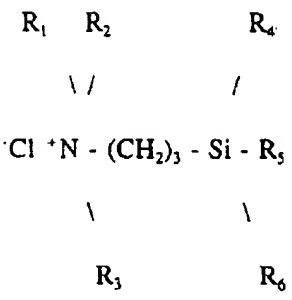
achieve appropriate penetration of said polymeric substrate while retaining the functional characteristics of said polymeric substrate.

6. A method accordingly to claim 5 wherein said solvent results in the desired degree of swelling of said polymeric substrate in less than ten minutes.

7. A method according to claim 5 wherein said solvent is ethyl acetate, a mixture of tetrahydrofuran, and methanol, or any organic solvent for said quaternary ammonium salt that swells said polymeric substrate.

8. A method for creating a catheter having non-leaching, antimicrobial properties comprising: providing a polymerizable or monomeric quaternary salt in a solvent; providing a catheter; and contacting said catheter to said solvent containing said quaternary salt such that said quaternary salt polymerizes within and upon the catheter in situ.

9. A method according to claim 8 wherein said quaternary salt has the general formula of:



10. A method according to claim 9 wherein R_1 and R_2 are methyl groups, R_3 is octadecyl, and R_4, R_5 and R_6 are methoxy groups.

11. A method according to claims 8, 9 or 10 wherein said polymerization of said quaternary salt is achieved by exposing it to 0.1 N NaOH, 0.1 N HCl, heat or a combination thereof.

12. A method according to claims 8, 9 or 10 wherein said solvents for said quaternary ammonium salt rapidly swell said catheter approximately 20-50 percent by weight in approximately 10 minutes or less while retaining the functional characteristics of said catheter.

13. A method according to claim 13 wherein said solvent is ethyl acetate, a mixture of tetrahydrofuran and methanol, or any organic solvent for said quaternary ammonium salt that swells said catheter.

14. A method according to claim 8 wherein said catheter is made of silicone, polyurethane, thermoplastic or plastic.

15. A method according to claim 8 wherein said catheter is impregnated with antibiotics.

16. A method for creating a non-leaching, biocompatible, antimicrobial, polymeric coating comprising: providing a polymerizable or monomeric quaternary salt in a solvent; exposing a substrate having interstices in which said quaternary salt can be absorbed and polymerized; permitting said quaternary salt to be absorbed by the substrate; and polymerizing said quaternary salt such that an interpenetrating network is formed within the interstices of the substrate.

C98-A17 Page 1

Textiles Having the Ability to Deliver Reactive Chemical Systems

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Annual Report, September 1999

Goal:

The goal of the work is to elucidate the fundamental considerations and demonstrate the techniques that can be used to develop textile materials which can be used to deliver reactive chemicals. In this demonstration, we have selected an application which has a significant commercial potential, the use of textiles to deliver antimicrobial activity. The methods of attachment and the general considerations should be similar for delivery of: catalysts in chemical synthesis, reactants to remove pollutants from air or water, and for producing materials with biocompatibility.

Abstract:

The work has demonstrated the following methods for incorporating reactive chemicals in textile materials:

1. Mechanistically understood chemical modification of the surface of fibers by the simple addition of reactive monomers to a reactive fiber.
2. Mechanistically understood creation of sites on an unreactive fiber, followed by chemical addition of reactive monomers.
3. Creation of reactive sites and addition of reactive monomers by high-energy photo-chemical or thermo-chemical processes where the mechanisms of grafting are not well defined.
4. Coating of textile surfaces by polymeric solids which are held in place physically or with reactive covalent links to the fiber surface.
5. Copolymerization of reactive compounds into fiber forming polymers followed by extrusion into fibers.

Textile materials created by each of these methods have been shown to be effective antimicrobials when tested against a typical infectious test bacteria. Some have also been shown to be effective against infectious protozoan species as well. Work is progressing on determining the actual, effective and maximum coverage of reactive chemical on the textile material. Work proposed on the kinetics of reaction/release of the active compounds has not yet begun.

Introduction:

When delivering reactive species from a fiber or fabric, several considerations. (1) The reactive chemical species must be attached to, or incorporated into the textile (fiber). (2) The reactive species must be held in a stable form until it is needed. (3) There must be a large reservoir of the reactive species, or the reactive species must be easily regenerable (*in situ*). (4) When needed, it must be readily available, and (5) The association with, or attachment to the textile must not interfere with the desired chemical action.

The durability (or rechargeability) must exceed the required or expected lifetime of the textile product. If the reactive species on the fiber or fabric can be recharged after it has been depleted, waste products can be reduced. In most cases, criteria (2) and (4) are opposing requirements which must be balanced. For example, the reactive species can be held within the polymer, and slowly diffuse to the surface. However, when needed, the surface concentration of the species will be rapidly depleted. Thereafter, a considerable lapse of time is required for more of the material to diffuse to the surface. There are two categories of antimicrobials – those that inhibit growth (bacteriostatic agents), and those that kill the organism (bacteriocidal agents) There are also differences in the amount of time required for specific chemicals to be effective. In general, the more reactive chemicals work quicker, but are also more likely to react with and be used up in competing chemicals that have nothing to do with the microbes that should be the target or their action.

In the area of antimicrobial products, there are the additional requirements for demonstrating effectiveness against a variety of harmful organisms. Since the products may be sold directly to retail consumers, there are questions of safety and toxicity to humans.

Some fundamental questions must also be considered in antimicrobial materials:
1. Is the reactive chemical released from the textile, or does it remain attached?
2. If released, what are the kinetics of release and what concentration is necessary to avoid depletion of the active chemical while in storage, before use?
3. What amount of water must be present for the antimicrobial activity to be effective?
4. How much of a reactive chemical can be accommodated on the textile surface? And how can it be measured?

An invited presentation prepared by some of the investigators was given at INDA Tec in Atlanta (9/99). It is available in the Conference Proceedings [1] It describes the chemical functionalities available for antimicrobial activity, the mechanism of action and a list of antimicrobial products that are available commercially in textiles.

Progress:

We have embarked on a combined theoretical and experimental analysis of this problem. We start with the idea that there must be a large reservoir of reactive species. Even if the species is regenerable, a large reservoir of reactive species is helpful. Since most fiber forming polymers (except cellulose) have very few reactive sites on their surface, it is impractical simply to chemically attach a significant amount of reactive species on their surfaces unless an amplification system is used. Although plasma treating can be used, it is too expensive for most applications.

Chemical degradation of the fiber surface to create reactive groups may be possible in the case of some "condensation" polymers. We have demonstrated this with one fiber and have then used the reactive groups on the surface of the fiber to attach an antimicrobial compound. The fiber produced has been shown to have a shelf life exceeding 6 months and is effective in killing a variety of organisms. Work is beginning on the maximum activity possible, the location of that activity (surface vs interior) and the kinetics of its decay in the absence of microbial challenge.

The work has also produced fibers with particles attached to their surfaces, either by physical attachment, or by covalent chemical attachment. One of the attached species has been shown to be an effective antimicrobial in fabric form. The other is still undergoing testing. Attachment of particles to fiber surfaces always suggests problems with durability, and durability studies are still needed. Again we have not yet determined the maximum loading of the textile with antimicrobial species, nor the level that is really needed. A light micrograph of highly colored particles attached to the textile surface is shown in Figure 1.

Figure 1 Particles Attached to Fibers

Scanning electron microscopy will be used with the uncolored, active compounds to examine the attachments to the fiber for strength of attachment and durability.

Another approach is to graft an active monomer onto the textile surface and allow some degree of polymerization to occur. We have had limited success in photo initiated grafting, and have yet to try chemically initiatiation, or initiation with hard radiation. All of these seem possible if a proper monomer is selected. As with all such grafting processes, one must determine the extent of graft vs homopolymer formation on the surface of the textile. The formation of homopolymer may lead to excessive stiffening or the textile or durability problems. Problems of surface wetting/spreading will have to be addressed to accomplish uniform, thin layers of active compound on textile surfaces. The uniformity of the "coatings" will be further studied by use of surface infrared techniques and EDAX in SEM.

A modification of this approach is to graft to the surface a polymeric material that contains groups to which the reactive species can be attached. *e.g.* poly(acrylic acid), poly(vinyl alcohol), poly(vinyl amine) or copolymers containing these groups. Again, there are several issues that need to be dealt with. (a) What is the maximum number of reactive groups that can be stored on the surface? (b) How can they be released? and (c) How does the mobility of the surface grafted polymer affect the delivery of the reactive species?

We consider (a) first. Although it would seem that the number of reactive species that could be stored by simply grafting a "storage polymer" to the surface of the fiber could increase without bound, we have found otherwise. When the radius of gyration of the storage polymer is equal to one-half the average distance between graft sites, the maximum storage potential is achieved. For smaller molecules, the surface has many bare spots which do not store any reactive species. When the storage polymer is larger than optimum, it blocks adjacent graft sites thus preventing attachment of additional storage polymers, again resulting in bare fiber surface. We are still investigating the effect of polydispersity, both in the storage polymer and in the graft-site density on the surface, on storage efficiency.

Next, let's consider (b). Here there are two possibilities. First, the reactive species must actually be released from the surface to be active. This will be controlled by the kinetics of the bond breakage in the reaction medium. We are currently examining how the kinetics are affected by the surface, much as fabric dyeing is affected by the surface. For this, we are grafting poly(acrylic acid) onto nylon 6,6 film. Next we neutralize the acid to differing extents and with different ions and then measure the surface conductivity. By comparing this data with similar data on dissolved poly(acrylic acid) salts, we will be able to determine the effect of the surface on the release of these ions.

The other approach is for the reactive species to perform its function while still attached to the surface. This would be the case if the reactive species acts as a catalyst. Its effectiveness will depend on how easily the reactants can approach and leave the reaction site, in much the same way that enzymes work. We will model this via molecular mechanics.

We are also attempting to measure and model how the mobility of the storage polymer changes upon grafting to the surface and how this effects the delivery of the reactive species or the approach of the reactants. This affects both (2c) and (3). Initially, we are measuring the surface conductivity of grafted poly(acrylic acid) salts as a function of temperature and frequency to measure the mobility of the storage polymer. It is expected that this mobility will be different from the bulk and from the solution forms of the storage polymer.

To accomplish these tasks, a surface conductivity cell has been constructed and is being calibrated over the frequency range 1 mHz – 100 kHz. A constant temperature and humidity chamber has been acquired and is being installed. Poly(acrylic acid) in a wide range of molecular weights have been acquired and are being grafted to nylon 6,6 film. Finally, the molecular mechanics modeling approaches are being refined. These will be combined to address each of the issues described above.

In working on antimicrobial textiles, we have had to address some issues of testing effectiveness. One standard test [2] places a treated textile on an inoculated agar plate. This test is suitable only when the antimicrobial agent can diffuse from the textile into the agar. A second method [3] places contaminated liquid on the surface of the fabric and measures the reduction in numbers of organisms as the result of that exposure. First there is the matter of exposure time which, as was pointed out, varies for different reactive agents. Then there is the matter of wetting. If the textile material does not readily absorb the contaminated liquid, the microbial kill rate will be much reduced. This problem was handled by sandwiching a small volume of the contaminated liquid between two layers of active fabric. A test method for wet filtration was previously developed in the laboratory of one of the investigators (DW). A test method for dry filters, which will aid in determining the necessity of water for antimicrobial effectiveness has yet to be addressed.

Continuing Work:

Additional needed work was indicted throughout the report, and concerns mostly the kinetics of the release of antimicrobial and of the reactions with microbes or other chemicals. Also, measurement of maximum and optimum fiber loading with antimicrobial agent is needed as well as the location and uniformity of the application.

References:

1. Broughton, Roy M., Worley, S. D., Cho, U., Lin, Jian, Sun, G., "Textiles with Antimicrobial Functionality," Conference Papers, INDA Tec 1999, Atlanta, GA, September, 1999, INDA, Cary NC.

2. AATCC Test Method 147-1993, "Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method," AATCC Technical Manual/1996, p 260-261, AATCC, Research Triangle Park, North Carolina.

3. AATCC Test Method 100-1993, "Antibacterial Finishes on Textile Materials Assessment of," AATCC Technical Manual/1995. p 148-149, AATCC, Research Triangle Park, North Carolina.

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Ga Tech:	Shuying Yang, GRA
Auburn:	Shi Wei Huang, GRA
	Unchin Cho, GRA
	Jian Lin, Postdoctoral Fellow

Industrial Contacts:

A number of industrial contacts were developed in the course of preparing the publication in Reference 1. Additional contacts were made as a result of the presentation. We estimate contacts with at least a dozen companies. Discussions have been very helpful to the investigators, and several of the companies have expressed a need for discussions and help, particularly in the area of testing for antimicrobial effectiveness.

Web Site:

Information concerning this project can be found at web site

www.eng.auburn.edu/department/te/ntc/indes.html

3. Cellulose Grafting: Past, Present and Future

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INTRODUCTION

Cellulosic graft copolymerization is of great interest with regard to the increased use of biobased materials. Indeed the graft copolymers are perhaps the clearest example of useful materials bringing together synthetic and natural polymers. These use biomass in all its many lignocellulosic varieties ranging from wood, grasses, and other plants to essentially pure celluloses such as cottons. Coupled with this aspect is the intrinsic biodegradability of the cellulosic component. The challenge of the Energy Conversion Utilization Program (ECUT) and similar programs is to expand and develop useful large scale and hi-tech smaller scale applications of such materials in an economic cost-effective manner. These objectives have not yet been achieved, and increased, well-programmed research is clearly and unequivocally needed. The desperate need to produce plastics, fibers, films, and other materials with biodegradability has given a continuation to such programs, which began with the need (now longer range) of replacing all or part of petroleum and natural gas feedstocks with renewable biomass. The need for a useful degree of photo and other types of degradability should also not be overlooked. This chapter will review the present status of such research, where the future emphasis should be stressed and the relative efforts currently under way in the United States compared with the overall international effort.

The author was privileged to be deeply involved in such research since July 1952, one year before the first conscious, deliberate, and successful synthesis of a cellulose graft copolymer was presented (Waltcher, Burroughs, and Jahn 1953). The preparation of this report was also based on presentations at major international meetings in 1988 (Tenth Cellulose Conference, Syracuse, N.Y., June; Cellucon '88, Kyoto, Japan, November; Nisshinbo Conference on Cellulose Utilization, Tokyo, Japan, December), and literature searches from January 1985 to December 1988 (including U.S. and foreign patents) to ensure adequate coverage of recent and present developments.

EARLIER RESEARCH - 1953-1984

Graft polymerization per se was first reported in 1946 (Carlin and Shakespeare). In 1943, however, vinyl and allyl esters of cellulose were prepared and copolymerized in experiments conducted with them. Although only crosslinked products resulted, certainly grafting must also have taken place (Ushakov 1943). Cellulose and its derivatives have been among the most popular as grafting substrates since 1952. Perceived perhaps as the first new type of cellulose derivatives, grafting was eagerly seized upon for research. By 1984, more than 1000 papers were published and patents granted on the subject. Grafting, which is often carried out heterogeneously, is an excellent method of modifying not only natural polymers but also polymers in fiber form. It was therefore even more attractive to carry out extensive work on the subject. This effort is still continuing. One excellent monograph (Hebeish and Guthrie 1981) and a number of rather thorough reviews (Krassig and Stannett 1965; Arthur 1970, 1985; Stannett and Hopfenberg 1971; Bhattacharya and Maldas 1984; Hon 1982; Samal, Sahoo, and Samataray 1986) on the subject have been published. These may be regarded as

key references. As with polymer science in general, research in cellulose grafts may be broken down into synthesis, characterization, properties, and applications.

Synthesis

Although ionic and condensation methods of grafting to cellulose have been briefly reported, the overwhelming majority of methods employed have been by free radical mechanisms. The general procedure is to generate free radicals on the cellulose molecule and then to introduce a vinyl or diene monomer. The macroradicals then initiate polymerization, forming graft copolymers. The various methods for carrying out this process via free radicals have been classified by Stannett and Hopfenberg (1971). Some notes and key references will be presented under similar headings.

Chain Transfer and Redox Methods

In these methods, a vinyl or diene monomer is polymerized in the presence of a cellulosic material. In the chain transfer method, the growing chain can then abstract hydrogen or another atom leaving behind the desired macroradical to initiate grafting. It is clear that the process is not too efficient and also leads to an equal amount of homopolymer. Nevertheless, it has a certain practicability and, in fact, similar methods are widely used industrially to produce heterogeneous grafts such as high-impact plastics. A useful development with this method is to introduce onto the cellulose groups that contain atoms that are readily extractable by free radicals. This was the first grafting method used, with halogens (Waltcher, Burroughs, and Jahn 1953). More recently, ethylene sulfide was reacted with cellulose to form mercaptan groups. These are very efficient chain-transfer agents. So far the growing chains from free radical polymerizations have been discussed as methods to generate the macroradicals. Radical transfer from the free radical catalysts themselves, although this is not strictly speaking chain transfer, can also be used with considerable success. To achieve this, the catalyst needs to be sorbed into the cellulose matrix itself. Potassium persulfate is a good example. A high concentration of the sulfate ion radicals are then formed, for instance, by heating persulfate, in the cellulose matrix itself. Abstraction can then compete successfully with initiation of homopolymer.

An even more efficient extension of the radical transfer approach is to sorb part of a two component redox system such as ferrous ions into cellulose. The monomer is then introduced with the second component such as hydrogen peroxide. Bridgeford has discussed such methods in detail (1962).

The xanthate method involves a redox system and has considerable promise for industrial exploitation. It was first reported by the Scott Paper Company in 1964 but still has not been commercialized. A number of pilot plants were built, however, and operated successfully. It is possible that the method will still be used on a large scale, but because it is tied to the xanthate rayon process, it has lost favor. However, xanthation can be carried out deliberately to low degrees of substitution. The xanthate group reacts with hydrogen peroxide to yield free radicals. The presence of ferric or ferrous ions may be necessary.

Direct Oxidation

A number of metallic ions have been used for the direct oxidation of cellulose to its macroradicals. The best known examples are ceric salts such as the sulfato and nitrate. The reaction is complex and not completely understood. It is known that the ceric ion forms a complex with cellulose hydroxyl groups in aqueous solution. The complex then dissociates in the presence of acids, into

a cellulose radical, a hydronium ion, and a cerous ion. The monomer is added either simultaneously or following pretreatment of the cellulose with a solution of the ceric salt. Initiation is rapid and efficient and in principle, leads to little homopolymer. In fact, some monomers themselves, notably acrylamide and acrylic acid, react with ceric ion to form radicals and homopolymers.

In addition to cerium (IV) salts, vanadium (V), manganese (III), cobalt (III), and chromium (VI) salts have been used. Other oxidizing agents such as permangates, bromates, and periodates also form radicals and initiate grafting. A considerable number of papers and patents have been published on the direct oxidation method and have been discussed and referenced in the various reviews (Hebeish and Guthrie 1981; Krassig and Stannett 1965; Arthur 1970, 1985; Stannett and Hopfenberg 1971; Bhattacharya and Maldas 1984; Hon 1982; Samal, Sahoo, and Samataray 1986).

Cellulose Initiators

Another general method of grafting to cellulose is to form chemically an initiator, such as a peroxide or hydroperoxide, on the macromolecule. This can then decompose into radicals and initiate graft copolymerization. If a reducing agent is used, the homopolymer formation can be largely eliminated. For example,

$$\text{Cell-OOH} + Fe^{2+} \longrightarrow \text{Cell-O}^{\cdot} + Fe^{3+} + OH^{-} \qquad (1)$$

Peroxides can be formed by ozonization, a method that has been studied chiefly in the Soviet Union, or by radiation of the substrate in air or in hydrogen peroxide solution. Similar reactions have been carried out with cellulose nitrate, ethylcellulose, and benzylcellulose. There is a danger with these methods that extensive degradation may occur, which has not been assessed adequately in the literature.

In addition to peroxides, other initiating groups can be introduced into the cellulose molecule, such as peroxy esters. Diazonium salts have also been synthesized and used as grafting initiators. As with the hydroperoxides, the addition of a reducing agent such as ferrous ammonium sulfate essentially eliminates the formation of homopolymers. The use of diazonium salts has also been extensively studied in the Soviet Union.

Cellulosic Comonomers

Perhaps one of the most obvious ways to synthesize graft copolymers is to form allyl or vinyl cellulose derivatives and then copolymerize with a suitable vinyl or similar type of monomer. This was indeed probably the first, although not a conscious attempt at grafting (Ushakov 1943). Unfortunately, at that time there was no knowledge of grafting and the degrees of substitution were presumably too high and only crosslinked products were produced. With low degrees of substitution, however, and a proper choice of monomer reactivity ratios, graft polymers without much homopolymer could be prepared by this technique. To our best knowledge, there has been little progress or research on this approach. This was the earliest and perhaps one of the most obvious ways to form cellulose graft copolymers, i.e., to form vinyl or allyl cellulose derivatives. Among the derivatives prepared have been cellulose allyl esters, cellulose methacrylates, and cellulose allyl ethers, as well as cellulose crotonates and maleates. More recently vinyl groups were introduced with cellulose nitrate by reacting with allyl monocarbonate and hexamethylene diisocyanate. Grafting to these derivatives can be accomplished by direct free-radical vinyl copolymerization. It can readily be seen that if there are several unsaturated groups on each cellulose molecule, heavy crosslinking will rapidly result. The situation is in many ways comparable to the polymerization of unsaturated polyesters. With low degrees

of substitution, however, or properly balanced monomer reactivity ratios, graft copolymers that are essentially free from crosslinks can be prepared by this technique.

Radiation Methods

Both ultraviolet light and high-energy radiation have been used to initiate grafting to cellulose and its derivatives. The latter method has, however, been by far the most thoroughly studied. In fact, high-energy radiation grafting has probably been investigated in more detail than any other method. Because the chemistry of the process is relatively obscure, radiation initiation has been used in experiments designed to reveal details of the grafting process itself. No chemical reagents are used normally for the radiation-grafting techniques, so it is essential to make the substrate accessible to the monomer. In fact, with a suitable swelling agent only negligible degrees of grafting are possible. Three methods developed for radiation grafting have been successfully used for cellulose.

Peroxide Method. The cellulose substrate is irradiated in air to form peroxide derivatives, which can later be used for grafting as previously described. This method has been studied less with cellulose than with many other polymers; however, preirradiation grafting in air has been frequently used. Here it seems clear that a combination of peroxide and trapped-radical initiation is operating. The grafting yields have been increased by first soaking the cellulose. In the case of viscose rayon fibers, for example, the fibers are soaked in hydrogen peroxide solution.

Preirradiation Method. This method involves irradiating the cellulosic substrate, preferably in the absence of air, and subsequently contacting the irradiated material with the monomer and swelling agent. Both liquid and vapor-phase grafting has been used, and nearly every kind of cellulosic substrate has been studied. Again, a suitable swelling agent is essential for successful grafting. In general, it is better to irradiate dry to produce the maximum number of free radicals and then to admit the monomer and swelling agent together. In the case of vapor phase grafting, the swelling agent can also be in the form of vapor, such as water vapor. In addition to water vapor, methanol and acetic acid vapors were found to be effective promoters of the grafting process. In liquid-phase preirradiation grafting, these additives were also found to be effective. Generally, the same monomer-swelling agent systems were effective for both the mutual and the preirradiation techniques. These will be discussed further in the section on mutual grafting methods. With the trapped-radical method as opposed to the peroxide method of preirradiation grafting, very little homopolymer is produced. A disadvantage, however, is that the degradation of the cellulosic backbone is usually greater with the preirradiation method. This is particularly true when the grafting is carried out in the presence of air or oxygen. Less degradation is encountered with the mutual method of irradiation because of the protective action of the vinyl monomers present during the actual irradiation. Preirradiation is often carried out in air, and the combination of peroxides and grafted radicals that results is used to initiate the grafting reaction. In spite of the possibly deleterious effects of the concurrent degradation, the preirradiatior method is very attractive economically. It has been used for pilot-plant studies of the grafting of styrene and other monomers to rayon.

Mutual Method. In this method, the cellulosic substrate is irradiated directly in contact with the monomer. The cellulosic material can be actually dissolved in the monomer or monomer-solvent mixture, or simply swollen. Either a vapor-phase or liquid-phase monomer can be used. The most usual technique, however, is the irradiation of the swollen cellulosic material in the liquid monomer or

monomer solution. It is clear that much homopolymer will also be generated by direct radiolysis of the monomer, the monomer-solvent, or the monomer-swelling agent mixture, but this can be successfully controlled. The cellulose acetate-styrene system has been studied in considerable detail, and methods have been developed for separating the homopolymers from true graft copolymers. It was apparent from the results of these studies that the degree of swelling of the cellulose substrate has a profound effect on both the yield of graft and the molecular weight of the grafted side chains.

Ultraviolet-Light Grafting

This method of direct radical formation and grafting has received comparatively little study, although the first experiments started as early as 1959. Cellulose derivatives have been investigated more than cellulose itself. The latter normally involves the addition of photosensitizers. In principle, the preirradiation and the mutual methods can be used. It is also clear that photochemical grafting can be useful for the surface modification of grafting. Hon has described some more recent experiments on ultraviolet grafting.

Other Methods of Free Radical Grafting

A number of methods have been developed to form graft (and block) cellulose copolymers. In general, they involve some mechanical breaking of the cellulosic chain such as mastication, vibratory milling, extrusion, or even ultrasonics and swelling techniques, in the presence of a vinyl monomer. Electrical discharge and plasma procedures have also been studied mainly for surface grafting.

The mechanical treatments together with grafting carried out simultaneously with certain pulping processes represent less well defined but in principle practical methods capable of being scaled up for industrial exploitation. Some key workers in this approach have been Young, Hon, and their coworkers (Young, Achmodi, and Barkalow 1985; Hon 1985).

Ionic Polymerization Methods

These were studied very little in the early period under review. Normally such methods involve the use of nonaqueous solvents, which are less attractive to the cellulose and allied industries than aqueous methods. Nevertheless, there were a number of approaches using anionic routes, which have been discussed rather fully in the key reviews. In general, shorter grafted side chains but rather higher degrees of substitution were obtained compared with free radical methods.

One very interesting cationic method has been described using boron trifluoride in nitrogen to form the cations, which were then reacted with isobutylene. Like the National Lead Process involving the Ziegler-Natta type of polymerization, it seems clear that little or no true grafting was obtained. However, in both cases the coated or encapsulated celluloses produced had excellent water resistance and other useful properties.

Ring Opening Methods

A number of reactive rings such as ethylene oxide, ethyleneimine, propiolactone, and ethylene and propylene sulfides have been reacted directly with cellulose following a suitable pretreatment. Generally, low-molecular-weight grafted side chains but relatively high degrees of substitution were obtained. Polyamides were successfully grafted to celluloses by reacting caprolactam with suitable cellulosic acid chlorides. Again, low DP grafts were obtained.

Condensation Methods

Methods of this type have received little study compared with the grafting of vinyl polymers. The most extensive studies have been made in the Soviet Union. A distinction can be made between adding an already formed polymer to a reactive functional group of the cellulosic material and using reactive groups on the cellulosic molecule to initiate condensation or ring-opening polymerization.

An example of preliminary work on the former method is the reaction of a low-molecular-weight poly(ethylene adipate) acid chloride with cellulose, using either a solvent-exchange or interfacial polymerization technique. A somewhat analogous study involved the reaction of telomers of poly(acrylic acid) having chlorine end groups with 6-aminoethylcellulose. Only a small proportion of telomer became attached. Alkali cellulose also did not react extensively with the poly(acrylic acid) telomer.

Direct polycondensation grafting of aminoenanthic acid chloride to cellulose and its derivatives has also been achieved. A low degree of substitution and molecular weight was found. An interfacial approach with cellulose xanthate gave similar results. In principle, the direct addition of a polymer with suitable functional groups could be attractive. However, the accessibility of one polymer to another except in solution and reactivity considerations has negated this method of synthesis.

In conclusion, it can be said that studies of methods of synthesis of cellulosic graft copolymers (mainly free radical in nature) dominated work through 1983. Comparatively little attention has been given to characterization, properties, and applications of such products.

Characterization of the Graft Copolymers

In addition to developing methods of grafting and examining their properties, it is necessary to characterize as closely as possible the pure grafts and to estimate the extent and molecular nature of the resulting graft. In some respects, cellulosic graft copolymers are rather easy to investigate because the solubilities of the two homopolymers and the grafts themselves are often quite different from one another. Furthermore, the cellulosic backbone can be destroyed by acid hydrolysis, and the molecular weight and other properties of the isolated side chains can be determined. This has made research on the structure of cellulosic grafts attractive not only for the grafts themselves but as models for grafting in general.

Whether the grafting reaction takes place in solution or within a swollen, insoluble cellulosic substrate, such as film or fiber, it seems inevitable that some homopolymers are present together with the graft and unreacted cellulose. In rather early work, the grafting of acrylamide to cellulose film was carried out by an ultraviolet-light technique. It was found that the graft copolymer, polyacrylamide, and the cellulose itself were all soluble in cuprammonium hydroxide. On acidifying, however, only the ungrafted cellulose and the graft copolymer precipitated. By weighing the precipitate, the amount of the grafting could be measured, but the amount of ungrafted cellulose could not be determined. This simple procedure was later applied to a number of other grafting methods. It was seen that each method of grafting gave different efficiencies of grafting and that the preirradiation technique was the most efficient.

With heterogeneous grafting to semicrystalline polymers, especially fibers such as cotton, the morphology and orientation can be changed. This, in turn, can greatly change the properties and will depend on the grafting method used and the choice of monomers and swelling agents and other additives. This aspect of

the structure-property relationships of cellulose graft copolymers has been studied in depth, particularly by Arthur and his coworkers (1985).

Properties

The properties of cellulosic materials--pulp and paper, textiles, and regenerated cellulose--and cellulose derivatives can be dramatically changed by graft copolymerization. Although data concerning properties and applications are scant in comparison with data concerning synthesis and physicochemical characterization, a sufficient technology has emerged to permit the beginning of a rational tailoring of properties in cellulosics via graft copolymerization.

Graft copolymerization has resulted in improvements in a wide variety of properties, including tensile strength; resistance to microbial degradation, abrasion, and acids; dye receptivity; wet strength of paper; and, adhesion. In addition, an entirely new spectrum of properties can be imparted, such as changing pulp, paper, cellophane, and fibers into ion-exchange materials including membranes by the controlled grafting of anion- and/or cation-exchange groups onto the cellulose. It has been demonstrated that moisture regain in cellulose and cellulose acetate can be reduced by controlled radiation-induced grafting of styrene. The water uptake can also be increased by grafting hydrophilic monomers including the synthesis of the so-called super water absorbing celluloses. It has also been demonstrated that the compatibility of dissimilar polymers can be markedly improved by adding small quantities of cellulosic graft copolymers "constructed" from the two incompatible backbones. The latter observation has considerable implications regarding the formation of stable and useful "polymer alloys," or "polyblends."

Cellulose itself is relatively inexpensive but cellulose derivatives are often expensive. Property improvements and alterations in properties achieved by grafting will usually be accompanied by an increase in cost. This immediately suggests that applications of some such end products will tend to be more specialized than those of the starting material.

It is recognized that the grafting process results in the formation not only of true covalently bonded graft copolymers but also of residual homopolymers of the substrate polymer and newly formed homopolymers corresponding in the chemical repeat unit to the grafted side chains. Many workers have scrupulously attempted to synthesize, isolate, and subsequently characterize the pure graft, whereas others, though acknowledging the presence of residual homopolymers, have proceeded to evaluate the end properties of the graft contaminated by the homopolymer impurities. Although a definitive study on the effect of homopolymer content in the properties of graft mixtures has not been made, it is cautioned that graft copolymers containing unextracted homopolymer will have properties that differ from those of a pure graft.

Many studies have been reported on the properties of grafted cellulose fibers--mainly on cotton and rayon, but also on jute and other natural fibers (Mohanty 1987, 1988). Such studies have included water moisture regain, resistance to soil burial, dyeability, mechanical, and thermal properties. Properties of grafted pulp and paper have also been extensively studied. All the property studies are well reported in the references and need not be repeated here. The subject of biodegradability will be discussed separately.

Applications

A considerable number of applications have been explored. In general, researchers have concentrated on synthesis, and to a lesser extent, characterization and properties rather than on developing suitable applications. For example, in the

excellent monograph on cellulosic graft copolymers by Hebeish and Guthrie (1981), only 16 pages out of 345 are devoted to applications. Nevertheless, there were 141 references through 1979, and there have been many more since then. In more recent years, increased attention has been given to this area. The subject of applications will be discussed in the present and future sections of this review. In general, there are applications to wood itself (Czikovsky 1968), to textile fibers (Arthur 1985), to pulp and paper (Phillips et al. 1972), and to membranes. There are also a number of miscellaneous applications. Some key references are given plus the reviews.

PRESENT SITUATION - 1985-1988

About 450 papers and patents were published in this 4-year period. Of these, about 350 were concerned with grafting to cellulose itself. As with the earlier years, synthesis and research on mechanisms and variations on established methods have been the major thrust. Recently, the effect of reaction variables on the composition of the grafts and the amounts of both homopolymers has been emphasized more than in earlier years. This research has inevitably included characterization and property studies. Together, however, the three areas of synthesis, characterization, and properties make up only about 62% of the published work. The remaining 38% was concerned with papers and patents discussing possible applications. This is in marked contrast to the earlier years when synthesis probably made up at least 75% of the total effort. The contrast was even greater in 1988.

In the area of synthesis, research into various features of the conventional free radical methods has continued. The effects of changes in the reaction variables on the yields, homopolymers and molecular weights of the side chains have been emphasized. The ceric ion and xanthate initiation techniques have dominated chemical methods. High energy radiation and photochemical grafting continued to be active areas of research. Studies on the latter method have increased markedly and it was by far the subject of the greatest number of publications on synthesis. This may be linked to a number of hi-tech types of imaging processes apparently under development.

There has also been a sharp increase in ionic grafting. Anionic grafting which links preformed living polymers to substituted celluloses has been explored in depth by Narayan and his coworkers (Biermann, Chung, and Narayan 1987; Narayan and Shay 1986). Their methods have the advantage of being able to achieve controlled degrees of substitution, molecular weights, and molecular weight distributions of the side chains. This is in marked contrast to the free radical techniques developed to date. At the moment, the need for rather dry solvent systems and cellulose derivative substrates make anionic grafting economically unattractive on a large scale except for specialized products. These anionic polymerization products could, however, be high value added materials. More importantly, at the moment, they have great value for exploring property-structure relationships of the grafts of well-defined structure. There have also been a few papers using cationic methods. Although it is difficult to envisage it being practical on a large scale, the cationic approach widens the range of side chain monomers available. One method, however, uses tosylated bleached kraft pulp to initiate the cationic grafting of 2-methyl oxazoline and is potentially more attractive for industrial exploitation (Cheradame, Ambo, and Gandini 1986).

Characterization methods have continued along similar lines to those developed in the earlier years. Somewhat more attention has been given, however, to improving the acid hydrolysis of the grafts to isolate the synthetic side chains for their characterization.

The property studies reported during the past few years have also continued along the lines developed during the previous years. But more emphasis has been given to water sorption, retention, and diffusion. There were also a few isolated studies on polymer blends used with compatibilizing grafts, on acid resistance, and on thermal and photo degradation. Various biological, bioactive, enzyme immobilization, antimicrobial, cell attachment, and related studies have been emphasized much more than in earlier years. Curiously, no studies of the biodegradability per se of the grafts were reported, in spite of its obvious importance.

Research into possible applications of cellulosic graft copolymers has been very active in the past 4 years' particularly in Japan. Most activity has centered on grafted cellulosic membranes for gas and other separation processes, including alcohol from water. Nearly 27% of the reports were on membranes and ion exchange materials. Applications based on water sorption were emphasized, particularly for ~~super~~ sorbing materials for uses ~~such as sanitary~~ napkins, tampons, and diapers, and for soil stabilization and other agricultural uses. Miscellaneous applications ~~such as for~~ bandages and other medical uses were also important. Equally emphasized ~~were applications for~~ the immobilization of enzymes, antimicrobials, and for hemostatic and biomaterial related uses including controlled drug release and biocompatibility.

Applications of the graft copolymers in the coatings industry have been investigated to a considerable degree to increase adhesion and to impart other useful properties. Applications in plastics were studied, including the use of grafts for compatibilizing blends, such as molding compositions and composites. Newer areas have been developed particularly in uses for diazoprinting, copying, and recording applications--some based on photosensitive materials; these were chiefly Japanese developments. Water soluble grafts and uses for the cosmetic industry have been reported. Applications to the textile and pulp and paper industries continued to be explored, but along similar lines to those developed in previous, years including waste water treatment.

Minor activities included the use of cellulosic grafts for adhesives, catalyst support systems, latex coagulating agents, oil absorbers, and foaming agents.

FUTURE RESEARCH NEEDS

There is a strong need for research in a number of areas, which include the following:

1. Much more work on the biodegradability of cellulosic graft copolymers is needed. The extraordinary fact is that in the past four years (through 1988) not a single paper concerning this problem appears to have been published[1]. Even across all the years of research into cellulosic graft copolymers, the enzymatic and microbial degradability of such copolymers have been studied to only a small extent. With available landfill capacity rapidly diminishing, the disposal of over 160 million tons of waste annually in the United States is emerging as a key environmental problem of the future. More than half the municipal solid waste stream is made

[1] Editor's note: The past year (1988-1989) has seen a number of attempts to measure the biodegradability of graft copolymers and composite materials. However, standard methods of analyses are not yet available, making the definition and assessment of the biodegradability of materials an area of intensive and extensive research (see chapter 8).

of paper-based and synthetic plastic materials, which biodegrade quite slowly in the environment. The use of grafting and other chemical means to enhance the degradability of this fraction of waste will allow faster "recycling" of the landfill volume and also serve to reduce urban litter.

The grafting of short chain moieties to cellulose to alter the rate of both the light-induced degradability and the biodegradability of the material is one such possibility. Sensitized photooxidation of cellulose has been extensively discussed in the literature. With judicious choice of functional groups, light-sensitive centers and even photooxidation catalysts might be incorporated into the cellulose structure. An allied field of interest is the chemical modification of the structure of cellulose itself to increase its degradability in the environment.

Plastics are practically non-biodegradable in the soil. Grafting a significant fraction of the relatively more biodegradable cellulose derivatives to the synthetic polymer is likely to enhance its biodegradability. Rapid microbial degradation of the grafted cellulose component will in turn generate a network of voids in the plastic matrix, weakening the structure and at least causing brittleness, which will decrease the harm the plastic material may cause to the environment.

Cellulose-synthetic polymer grafting reactions have been studied by Narayan and others (see Chapter 7). Further research based on these pioneering studies may extend these techniques to achieve high levels of grafting in slurry (or solid reactant) systems in a continuous, fast process.

2. With all the work on methods of grafting, the effect of the lignin content on grafting yields and the characteristics and properties of the resulting grafts have been little studied. Most of the research has concentrated on pure forms of cellulose and its derivatives. More work is needed on this subject. The direct use of whole wood, steam-exploded woods, and high-yield pulps as grafting substrates is clearly of considerable industrial importance. Some such studies are under way by Glasser and others.

   Some work has been reported, particularly by Kokta and coworkers (see Chapter 2), on the effects of lignin on grafting. Coupled with this aspect, the possibility of grafting during the pulping process or on whole wood should be further explored. An important example is the work of Young, Rowell and colleagues.

3. Work on the super water-absorbing cellulose grafts needs to be expanded. Methods of reducing the effects of salts on the water sorbency and retention need to be explored, but an initial finding of Salamone et al. (1985) is a good start on the problem. The biodegradability of the "supersorbers" is also important and should lead to a new emphasis on cellulose and starch based sorbents compared with the overwhelming use of the purely synthetic polymers currently on the market.

4. Finally, cost-effectiveness of the resulting products are all-important. A reasonable economic analysis of the various methods of grafting with various substrates is needed. Even if somewhat tentative and speculative, such a study would be most helpful for the further development of grafting technology. The three subjects summarized above represent examples that could benefit from an economic analysis.

INTERNATIONAL ASPECTS

An analysis of the research reported during the past four years, up to December 1988, showed that 28 countries had published work on cellulose grafting. Japan led the way with 50 papers and patents followed by the Soviet Union with 40 entries and the United States with 33 published works. Other active countries are Egypt, Czechoslovakia, and Canada, but these all have less than 15 entries each.

REFERENCES

Arthur, J. C., Adv. in Macromol. Sci., Vol. 2, Academic Press, New York (1970) pp. 1-87.

Arthur, J. C., Encyclopedia of Polymer Science and Engineering, 2nd Edition, Vol. 3, Wiley-Interscience Publ., New York (1985) pp. 68-86.

Bhattacharya, S. N. and Maldas, D., Prog. Polym. Sci., 10 (1984) 171-270.

Biermann, C. J., Chung, J. B., and Narayan, R., Macromol., 20 (1987) 954.

Bridgeford, D. J., Ind. Eng. Chem., Prod. Res. Develop., 1 (1962) 45.

Carlin, R. B. and Shakespeare, N., J. Amer. Chem. Soc., 68 (1946) 876.
Cheradame, H., Ambo, T. A., and Gandini, A., Makromol. Chem., Macromol. Symp., 6 (1986) 261.

Czikovsky, Atomic Energy Review, (IAEA-Vienna), 6 (1968) 3.

Hebeish, A. and Guthrie, J. T., The Chemistry and Technology of Cellulosic Copolymers, Springer-Verlag, Berlin, 1981.

Hon, David N. S., ed., ACS Symposium Series, No. 187: Graft Copolymerization of Lignocellulose Fibers, ACS Division of Cellulose, Paper, and Textile Chemistry at the 182nd Meeting of the American Chemical Society, New York, NY, August 23-28, 1982.

Hon, D.N.S., "Mechanochemistry of Cellulosic Materials," Chapter 6 in Cellulose and Its Derivatives, J. F. Kennedy, G. O. Phillips, D. J. Wedlock, and P. A. Williams, eds., Ellis Horwood Ltd., Chichester, (1985) 71-86.

Krassig, H. A. and Stannett, V. T., Adv. Polym. Sci., 4 (1965) 111-156.

Mohanty, A. K., J. Macromol. Sci. - Reviews, C27 (1987-8) 593-639.

Narayan, R. and Shay, M., in Renewable Resource Materials, New Polymer Sources, C. E. Carraher, Jr. and L. H. Sperling, eds., Plenum, New York (1986) 137-146; Polym. Sci. Tech., 33 (1986) 137-146.

Phillips, R. B., Quere, J., Guiroy, G., and Stannett, V. T., Tappi, 55 (1972) 858.

Salamone, J. C., Rodriguez, E. L., Lin, K. C., Quach, L., Watterson, A. C., and Ahmed, I., Polymer, 26 (1985) 1234.

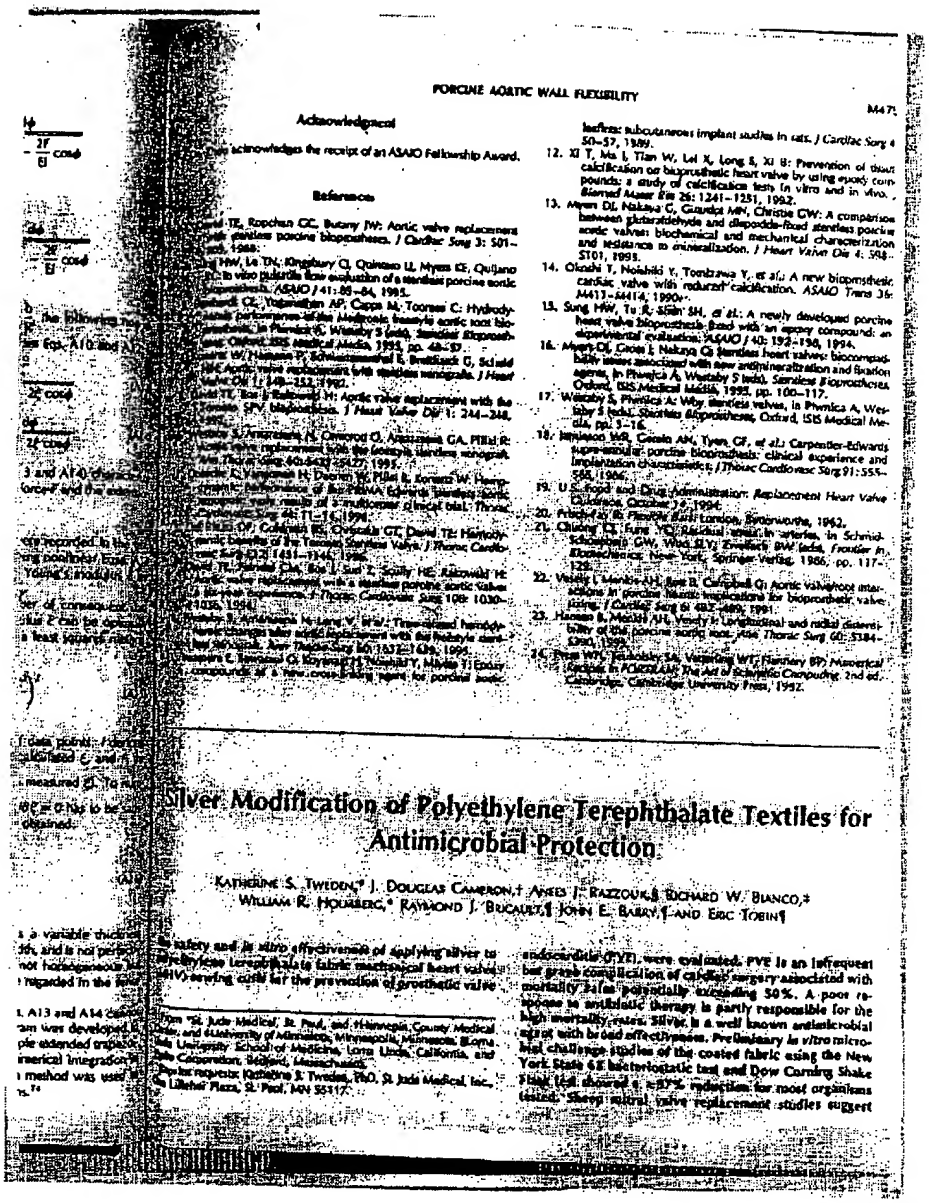
Samal, R. K., Sahoo, P. K., and Samataray, J., Macromol. Sci. Review, C26 (1986) 81-141.

Stannett, V. T. and Kopfenberg, H. B., Chapter XVII in Cellulose and Cellulose Derivatives, Part V, Wiley-Interscience Publ., New York (1971) pp. 907-936.

Ushakov, S. N., Fiz-Mat. Nauk., 1 (USSR) (1943) 35.

Waltcher, I., Burroughs, R., and Jahn, E. C., I.U.P.A.C. Conference, Stockholm (1953).

Young, R. A., Achmodi, S., and Barkalow, D., "Direct Modification of Cellulose in Woody Biomass and Sludge," Chapter 37 in Cellulose and Its Derivatives, J. F. Kennedy, G. O. Phillips, D. J. Wedlock, and P. A. Williams, eds., Ellis Horwood Ltd., Chichester (1985) 417-424.



PORCINE AORTIC WALL FLEXIBILITY M471

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References

[illegible] TE, Ropchan GC, Bussey JW: Aortic valve replacement [illegible] porcine bioprostheses. *J Cardiac Surg* 3: 501–[illegible], 1988.

[illegible] HW, Le TN, Klingbury O, Quinzeo LI, Myers KE, Quijano [illegible]: In vitro pulsatile flow evaluation of a stentless porcine aortic [illegible]. *ASAIO J* 41:80–84, 1995.

[illegible] CL, Yoganathan AP, Capps M, Toorsee C: Hydrodynamic performance of the Medtronic Freestyle aortic root bioprosthesis. In Fernicola JC, Westaby S (eds), *Stentless Bioprosthesis*. Oxford, ISIS Medical Media, 1995, pp. 48–57.

[illegible] W, Nilsson KJ, Schmiedtbauer E, Breitbach G, Schild [illegible]: Aortic valve replacement with stentless xenografts. *J Heart Valve Dis* 1: 148–152, 1992.

[illegible] TE, Bos J, Ballcomb H: Aortic valve replacement with the Toronto SPV bioprosthesis. *J Heart Valve Dis* 1: 244–248, 1992.

[illegible] S, Hirotsawa N, Cimmond G, Abbasoglu GA, Pillai R: Aortic valve replacement with the Freestyle stentless xenograft. *Ann Thorac Surg* 60:S422–S427, 1995.

[illegible] C, Vanermen H, Daenen W, Pillai R, Konertz W, Pomp-[illegible]: Hemodynamic performance of the PRIMA Edwards stentless aortic xenograft: early results of a multicenter clinical trial. *Thorac Cardiovasc Surg* 43: 71–76, 1995.

[illegible] OF, Caldwell RL, Cressaty GT, David TE: Hemodynamic benefits of the Toronto Stentless Valve. *J Thorac Cardiovasc Surg* 107:1431–1441, 1994.

[illegible] TE, Salerno CTA, Bos J, Lee T, Scully HE, Kaszowski H: [illegible] replacement with a stentless porcine aortic valve bioprosthesis. *J Thorac Cardiovasc Surg* 108: 1030–1038, 1994.

[illegible] S, Hirotsawa N, Ivanov V, Pillai: Time-related hemodynamic changes after aortic valve replacement with the Freestyle stentless bioprosthesis. *Ann Thorac Surg* 60:1632–1634, 1995.

[illegible] C, Bergmann G, Kleyman T, Nishida Y, White TJ: Evaluation of compounds of a new cross-linking agent for porcine aortic leaflets: subcutaneous implant studies in rats. *J Cardiac Surg* 4: 50–57, 1989.

12. Xi T, Ma L, Tian W, Lei X, Long S, Xi B: Prevention of tissue calcification on bioprosthetic heart valve by using epoxy compounds: a study of calcification tests in vitro and in vivo. *Biomed Mater Res* 26: 1241–1251, 1992.
13. Meyer DL, Nakaya C, Gourdjet MH, Christie GW: A comparison between glutaraldehyde and diepoxide-fixed stentless porcine aortic valves: biochemical and mechanical characterization and resistance to mineralization. *J Heart Valve Dis* 4: S98–S101, 1995.
14. Okoshi T, Nishibiki K, Tomizawa Y, *et al.*: A new bioprosthetic cardiac valve with reduced calcification. *ASAIO Trans* 36: M413–M414, 1990.
15. Sung HW, Tu R, Shin SH, *et al.*: A newly developed porcine heart valve bioprosthesis fixed with an epoxy compound: an experimental evaluation. *ASAIO J* 40: 192–198, 1994.
16. Meyer DL, Gross J, Nakaya C: Stentless heart valves: biocompatibility issues associated with new antimineralization and fixation agents. In Fernicola A, Westaby S (eds), *Stentless Bioprosthesis*. Oxford, ISIS Medical Media, 1995, pp. 100–117.
17. Westaby S, Fernicola A: Why stentless valves. In Fernicola A, Westaby S (eds): *Stentless Bioprosthesis*. Oxford, ISIS Medical Media, pp. 5–16.
18. Jamieson WR, Casale AN, Tyan GF, *et al.*: Carpentier-Edwards supra-annular porcine bioprosthesis: clinical experience and implantation characteristics. *J Thorac Cardiovasc Surg* 91:555–565, 1986.
19. U.S. Food and Drug Administration: *Replacement Heart Valve Guidance*, October 1, 1994.
20. Prichard AJ: *Flexible Shaft*. London, Butterworths, 1962.
21. Chuong CJ, Fung YC: Residual stress in arteries. In Schmid-Schonbein GW, Woo SLY, Zweifach BW (eds), *Frontier in Biomechanics*. New York, Springer-Verlag, 1986, pp. 117–129.
22. Vesely I, Mackin EH, Bee B, Campbell G: Aortic valvefoot interactions in porcine heart: implications for bioprosthetic valve fixing. *J Cardiac Surg* 6: 487–499, 1991.
23. Hansen B, Meuhl AH, Vesely I: Geometrical and radial distensibility of the porcine aortic root. *Ann Thorac Surg* 60: S184–S189, 1995.
24. Press WH, Teukolsky SA, Vetterling WT, Flannery BP: *Numerical Recipes in FORTRAN: The Art of Scientific Computing*. 2nd ed. Cambridge, Cambridge University Press, 1992.

Silver Modification of Polyethylene Terephthalate Textiles for Antimicrobial Protection

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The safety and *in vitro* effectiveness of applying silver to polyethylene terephthalate fabric mechanical heart valve (MHV) sewing cuffs for the prevention of prosthetic valve endocarditis (PVE), were examined. PVE is an infrequent but grave complication of cardiac surgery associated with mortality rates potentially exceeding 50%. A poor response to antibiotic therapy is partly responsible for the high mortality rates. Silver is a well known antimicrobial agent with broad effectiveness. Preliminary *in vitro* microbial challenge studies of the coated fabric using the New York State 66 bacteriostatic test and Dow Corning Shake Flask test showed a >99% reduction for most organisms tested. Sheep mitral valve replacement studies suggest

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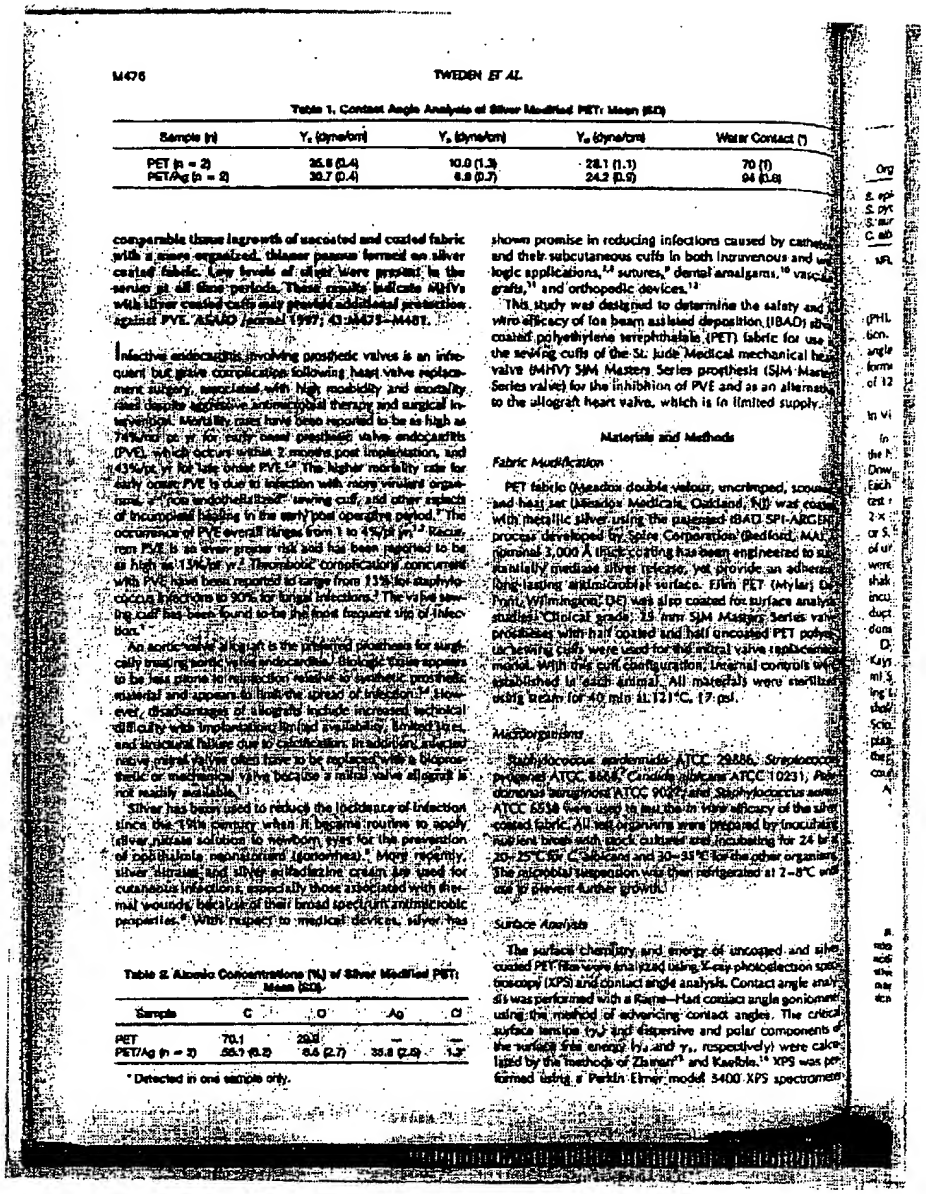


Table 1. Contact Angle Analysis of Silver Modified PET: Mean (SD)

Sample (n)	γ_s (dyne/cm)	γ_s^d (dyne/cm)	γ_s^p (dyne/cm)	Water Contact (°)
PET (n = 2)	35.8 (0.4)	10.0 (1.3)	28.1 (1.1)	70 (1)
PET/Ag (n = 2)	30.7 (0.4)	6.8 (0.7)	24.2 (0.9)	84 (0.8)

comparable tissue ingrowth of uncoated and coated fabric with a more organized, thinner pannus formed on silver coated fabric. Low levels of silver were present in the serum at all time periods. These results indicate sHAVs with silver coated cuffs may provide additional protection against PVE. ASAIO Journal 1997; 43:M471–M481.

Infective endocarditis involving prosthetic valves is an infrequent but grave complication following heart valve replacement surgery, associated with high morbidity and mortality rates despite aggressive antimicrobial therapy and surgical intervention. Mortality rates have been reported to be as high as 74%/pt. yr. for early onset prosthetic valve endocarditis (PVE), which occurs within 2 months post implantation, and 43%/pt. yr for late onset PVE.[1,2] The higher mortality rate for early onset PVE is due to infection with more virulent organisms, a "non endothelialized" sewing cuff, and other aspects of incompleted healing in the early post operative period.[3] The occurrence of PVE overall ranges from 1 to 4%/pt yr.[1,2] Recurrent PVE is at even greater risk and has been reported to be as high as 13%/pt yr.[2] Thromboic complications concurrent with PVE have been reported to range from 13% for staphylococcus infections to 90% for fungal infections.[3] The valve sewing cuff has been found to be the most frequent site of infection.[4–6]

An aortic valve allograft is the preferred prosthesis for surgically treating aortic valve endocarditis.[7] Biologic tissue appears to be less prone to reinfection relative to synthetic prosthetic material and appears to limit the spread of infection.[8] However, disadvantages of allografts include increased technical difficulty with implantation, limited availability, limited lifespan, and structural failure due to calcification. In addition, anechoic native mitral valves often have to be replaced with a bioprosthetic or mechanical valve because a mitral valve allograft is not readily available.[7]

Silver has been used to reduce the incidence of infection since the 19th century when it became routine to apply silver nitrate solution to newborn eyes for the prevention of ophthalmia neonatorum (gonorrhea).[9] More recently, silver nitrate and silver sulfadiazine cream are used for cutaneous infections, especially those associated with thermal wounds, because of their broad spectrum antimicrobic properties.[9] With respect to medical devices, silver has shown promise in reducing infections caused by catheters and their subcutaneous cuffs in both intravenous and urologic applications,[2,4] sutures,[9] dental amalgams,[10] vascular grafts,[11] and orthopedic devices.[12]

This study was designed to determine the safety and in vitro efficacy of ion beam assisted deposition (IBAD) silver coated polyethylene terephthalate (PET) fabric for use as the sewing cuffs of the St. Jude Medical mechanical heart valve (MHV) SJM Masters Series prosthesis (SJM Master Series valve) for the inhibition of PVE and as an alternative to the allograft heart valve, which is in limited supply.

Table 2. Atomic Concentrations (%) of Silver Modified PET: Mean (SD)

Sample	C	O	Ag	Cl
PET	70.1	29.9	—	—
PET/Ag (n = 3)	55.7 (6.2)	8.6 (2.7)	35.8 (2.9)	1.3*

* Detected in one sample only.

Materials and Methods

Fabric Modification

PET fabric (Meadox double velour, uncrimped, scoured and heat set (Meadox Medicals, Oakland, NJ) was coated with metallic silver using the patented IBAD SPI-ARGENT process developed by Spire Corporation (Bedford, MA). Approximately 3,000 Å thick coating has been engineered to substantially mediate silver release, yet provide an adherent, long-lasting antimicrobial surface. Film PET (Mylar) (DuPont, Wilmington, DE) was also coated for surface analysis studies. Clinical grade, 23 mm SJM Masters Series valve prostheses with half coated and half uncoated PET polyester sewing cuffs were used for the mitral valve replacement model. With this cuff configuration, internal controls were established in each animal. All materials were sterilized using steam for 40 min at 121°C, 17 psi.

Microorganisms

Staphylococcus epidermidis ATCC 29886, *Streptococcus pyogenes* ATCC 8668, *Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 6538 were used to test the in vitro efficacy of the silver coated fabric. All test organisms were prepared by inoculating nutrient broth with stock cultures and incubating for 24 hr at 20–25°C for *C. albicans* and 30–35°C for the other organisms. The microbial suspension was then refrigerated at 2–8°C until use to prevent further growth.

Surface Analysis

The surface chemistry and energy of uncoated and silver coated PET films were analyzed using X-ray photoelection spectroscopy (XPS) and contact angle analysis. Contact angle analysis was performed with a Rame–Hart contact angle goniometer using the method of advancing contact angles. The critical surface tension (γ_c) and dispersive and polar components of the surface free energy (γ_s^d and γ_s^p, respectively) were calculated by the methods of Zisman[13] and Kaelble.[14] XPS was performed using a Perkin Elmer model 5400 XPS spectrometer

Table 3. Microbial Challenges: Mean % Reduction

	NYS63		Dow Shake	
Organism	PET	PET/Ag	PET	PET/Ag
S. epidermidis	NR	99.0 (1.0)	12.5 (17.7)	97.1 (3.3)
P. aeruginosa	NR	98.7 (0.3)	NR	69.5 (43.8)
S. aureus	NR	98.2 (1.2)	NR	96.7
C. albicans	99.2 (1.8)	99.5 (0.2)	NR	98.6 (1.7)

NR no reduction.

Eden Prairie, MN) with monochromatic Al Kα x-ray radiation. Average depth of penetration was 60 Å and the take-off angle was 45°. Depth profiling to 400 Å depth was also performed using a 4 keV Ar^+ ion beam at a sputter rate for silver of 20 Å/min.

In Vitro Efficacy

In vitro efficacy of the silver coated fabric was assessed using the New York State (NYS) 63 test for bacteriostatic activity, the Dow Corning Shake-Flask test, and a static incubation test. Each assay was repeated on two separate days. The NYS63 test assessed for bacteriostatic activity consisted of inoculating 2×10^6 to 1×10^8 CFU of *S. aureus*, *S. progenes*, *C. albicans*, or *S. epidermidis* in a humidified atmosphere with 1 in squares of uncoated and coated fabric at 37°C for 24 hr. The samples were diluted with 99 ml of Letheen Broth and vigorously shaken for 1 min. Serial dilutions were plated on nutrient agar, incubated again for 24–48 hr and counted. The percent reduction of each organism was determined. Five replicates were run in each assay.

The Shake-Flask test consisted of inoculating 70 ml of Dow-Bob phosphate buffer with 3 ml of $1–2 \times 10^6$ CFU of *S. aureus*, *S. progenes*, *C. albicans*, or *S. epidermidis*, placing 0.75 g of uncoated or coated fabric in the suspension, and shaking vigorously for 1 hr in a wrist action shaker (Burrell Scientific, Pittsburgh, PA) at 37°C. Aliquots of solutions are plated into petri dishes and incubated 60 at least 24 hr and then counted. A percent reduction was calculated. An initial count was obtained before the samples were added.

A final bioactivity assay consisted of incubating 1×1 cm^2 pieces of coated and uncoated fabric for 48 hr at 30–35°C in 10 ml of tryptic soy broth inoculated with 10–100 CFU of either *S. aureus*, *P. aeruginosa*, or *S. epidermidis*. Specimens were rinsed in saline, fixed in 2% buffered glutaraldehyde, serially dehydrated with ethanol, dried with hexamethyldisilazane, coated with a gold palladium film, and imaged using scanning electron microscopy (Hitachi 450; Hitachi, Tokyo, Japan) to assess the nature of organism interaction with the fabric.

Silver Leaching

The leach rate of silver from fabric sewing cuffs was tested *in vitro* in serum. Two 17 mm valves with uncoated cuffs and two 17 mm valves with coated cuffs were exposed to 495 ml fetal bovine serum (Hyclone Laboratories, Logan, UT) for 7 days at 37°C with rotation at 100 rpm (Environ Shaker; Lab-Line, Melrose Park, IL). Five milliliter samples of the serum were subjected to elemental analysis for silver pre-exposure and at 5 hr, 1, 2, 3, 4, and 7 days post exposure. Elemental analysis was performed using an inductively coupled plasma atomic emission spectrometer (Atom Scan 16; Thermo Jarrell Ash, Franklin, MA). Standard curves were prepared using 10,000 ppm NIST Silver in 14% nitric acid. The range was from 0 to 500 ppb. All samples were lyophilized and then hydrolyzed in 17.5% nitric acid at 115°C for 24 hr. The samples were diluted to 14% nitric acid before analysis. The quantitation limit was 25 ppb.

Cardiac Valve Replacement (CVR) Model

All animal care complied with the Principles of Laboratory Animal Care and The Guide for the Care and Use of Laboratory

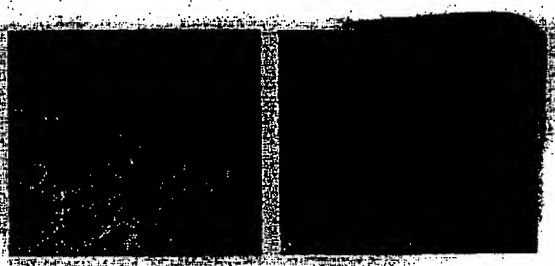


Figure 1.—Scanning electron micrographs of *S. epidermidis* adhesion to uncoated fabric (a) and silver coated fabric (b). Original magnification ×3,000. Magnification bar = 2.8 μm.

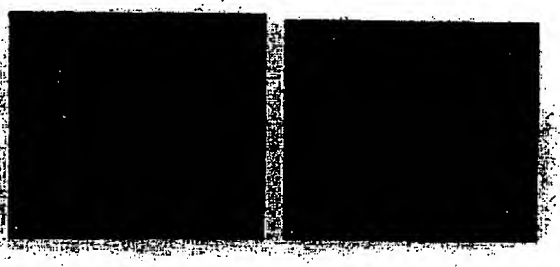


Figure 2. Scanning electron micrographs of *P. aeruginosa* adhesion to uncoated fabric (a) and silver coated fabric (b). Original magnification ×5,000. Magnification bar = 2.5 μm.

Animals (NIH Publication No. 85-23, 1985). All protocols were approved by the facility's animal care committee. 3M Mosaic bovine valves with full silver coated and full uncoated PET cuffs were implanted in the mitral position of four Suffolk sheep, each weighing ~25 kg. The native mitral valve was either left intact or the anterior leaflet was removed. No anticoagulants were given postoperatively. Prophylactic antibiotics were given for 7–10 days postoperatively. The valves were sutured into the annulus using interrupted horizontal mattress sutures of 3-0 Tevdek (Deknatel) with or without Teflon felt reinforcing pledgets. Serum samples were prepared from whole blood drawn pre-implantation, 1, 2, and 3 weeks post operatively, and pre-sacrifice and assayed for silver using elemental analysis as described above. The silver quantitation limit was 12.5 ppb. Values that fell below this limit were assigned the value 12.5 ppb. Four to five weeks post implantation, the valves were explanted after the animals were systemically anticoagulated with 300 units heparin/kg body wt and killed. Pre-operative, intraoperative, and post-operative techniques and explantation methods are described in detail elsewhere.[7] Explanted valves were fixed in McDowell–Trump fixative after rinsing with 0.1 M phosphate buffer and analyzed macroscopically and histologically for tissue reaction. Silver coated and uncoated sewing cuff histology samples were retrieved 45° from the two seams. The thickness of the tissue (pannus) covering the sewing cuffs was measured using a calibrated reticle.

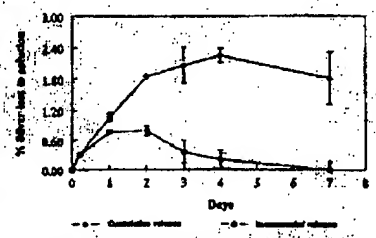


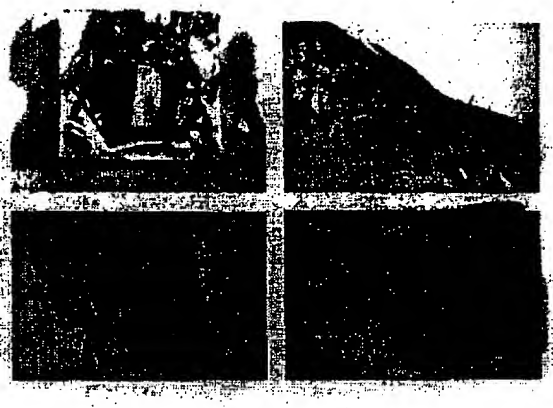
Figure 3. Cumulative and incremental in vitro leaching of silver from mechanical heart valves with ion beam assisted deposition silver coated cuffs in serum. Error bars are SEMs. Values were negative and was assigned 0.

Results

Surface energy and XPS measurements are shown in Tables 1 and 2. Modification of the PET film with IBAD silver resulted in a surface with increased hydrophobicity, decreased γ_s, and decreased polarity. XPS results show a surface that is predominantly C, Ag, and O. Analysis of the oxygen surface chemistry shows the oxygen is bound to C and not Ag. Depth profiling data shows that both C and O drop off after no more than 16 Å is removed (data not shown).

The results of the NYS63 and Dow Shake Flask tests are shown in Table 3. The mean percent reduction of organisms on the silver coated fabric in the static NYS63 test ranged from 98.2% for *S. aureus* to 99.2% for *S. pyogenes* compared with little to no reduction with the uncoated fabric. The Dow Shake test showed a mean reduction that ranged from 88.5% for *S. pyogenes* to 98.6% for *S. aureus* for silver coated fabric compared with little to no reduction with the uncoated fabric. *S. pyogenes'* apparent need to be in prolonged direct contact with silver to achieve inhibition may explain the difference in reduction in the Dow Shake test compared to the NYS63 test. The scanning electron microscopic analysis of uncoated and silver coated fabric incubated with microorganisms indicated that there were fewer organisms attached and less colonization of the bacteria on the silver coated fabric than the uncoated control for both *S. epidermidis* and *P. aeruginosa* (Figures 1 and 2). The silver coating on the fabric also inhibited the accumulation of *S. aureus*; most bacteria were attached as singlets or doublets (data not shown).

Cumulative and incremental *in vitro* silver leaching data are shown in Figure 3. The result show that ~2% of the silver is released from the cuff in serum within 7 days and the release has reached a plateau. The incremental release data show that the majority of the silver was released in the first 3 days. No silver was detected in uncoated controls, as anticipated. The maximum level of silver detected in the serum was 187 ppb (data not shown), which corresponds to 55 ppb in an adult human of normal blood volume (5 l). Ninety percent of silver



...ingested or absorbed is excreted in the bile,[9] which, in this case, would result in a modest 3.5 ppb serum silver level.

...histological gross macrographic and histology from the CVE study are shown in Figure 4. The gross reaction to the ...was similar for both the uncoated and the coated halves (Figure 4A). Grossly, the pannus was >75% healed at the 4 to 5 week time period. Histopathologic analyses showed that the degrees of foreign body giant cell (FBGC) response and fibrous integration of the two halves were similar (Figure 4). with an average score of 1.25 (FBGC) and 1.5 (fibrous integration) on a scale of 0–4, 0 being none and 4 being marked. Also, there was a suggestion that pannus maturity was more advanced on the silver coated side than the uncoated side. The pannus in the control areas was characterized by randomly oriented, "activated fibroblasts" forming a non lamellar pattern (Figure 4B). Dystrophic calcification was found in control areas only (Figure 4C). The pannus in coated areas was characterized by regularly oriented "mature fibroblasts" forming a lamellar pattern (Figure 4D). The mean pannus thickness of the uncoated half was 400 ± 251 μm compared with 269 ± 235 μm for the silver coated half. Serum silver levels are shown in Figure 5. A slight peak is suggested at two weeks, after which it dropped to below quantitation by death (4–5 weeks).

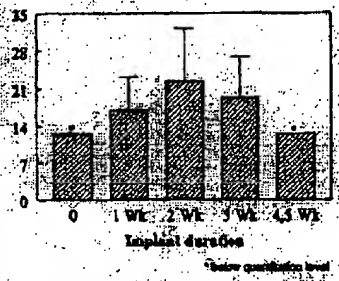


Figure 5. Serum silver levels in sheep with mechanical heart valves with half coated and half uncoated polyethylene terephthalate sewing cuffs.

Discussion

Silver salts and colloids have a broad spectrum and long history as antimicrobials. However, silver must be carefully administered to avoid silver toxicity syndrome associated as argyria, skin pigmentation, leukopenia, and kidney, liver, and neurologic tissue toxicity. The lowest serum level reported for this syndrome is 300 ppb.[9] Promising results have been published on the use of metallic silver and silver oxide coatings on vascular devices for the prevention of device associated infection.[9,10] Silver in these forms dissolves slowly providing protection against silver toxicity while offering surface contact antimicrobial benefits. Our in vivo CVE and in vitro leach studies indicate silver oxidation from PET fabrics to be far below toxic levels. XPS surface chemical analysis of the coating confirms the presence of a metallic silver state, with a thin layer of organic contaminant on the outermost surface. The XPS profile, together with the more hydrophobic and nonpolar nature of the coated surface, suggest that the IBAD process may have facilitated a surface coating of PET fragments with its hydrophobic groups (benzyl or methyl) outermost.

Our results support the broad spectrum antimicrobial activ-

ity of IBAD silver that others have reported for silver in general. IBAD silver inhibited the colonization on contact of gram positive organisms, a gram negative organism, and a fungus, all of which are implicated in PVE. Silver is postulated to inhibit microorganisms by binding to microbial DNA and subsequently preventing replication and by binding to the sulfhydryl groups of key metabolic enzymes resulting in denaturation and inactivation of those enzymes.[16,17] Although silver ion is considered one of the most potent heavy metal inhibitors of enzymes and microorganisms, its toxicity to higher organisms and humans is small because of its slow absorption and ease of reaction with chloride ion, proteins, and sulfhydryl groups.[18]

Metallic silver has been reported to be relatively nontoxic and inert to mammalian tissues, which is confirmed in this study. Tissue ingrowth was not inhibited in this application, and there was a suggestion that pannus organization was more advanced on the silver-coated fabric. A fully organized pannus would provide protection from cuff associated thrombosis and infection. The relative blood compatibility of silver is supported in this study and by other groups who have reported on its use in hemodialysis catheter and vascular graft applications.[19,20] Bambauer et al.[20] reported a decrease in platelet attachment and thrombus formation on silver-coated silicone rubber catheters for dialysis applications. Silver has also been shown to adsorb high levels of albumin, which perhaps contributes to its relatively good blood compatibility.[21]

The heart valve prosthesis sewing cuff evaluated is designed to aggressively promote tissue ingrowth with its rough, double velour texture. However, the rough morphology and the PET polymer itself are highly attractive to microorganisms involved in endocarditis as supported by the clinical finding that infections begins in the sewing cuffs of MHV prosthesis.[1] It has been suggested that intra contamination leading to early onset PVE occurs intraoperatively.[2] One group found that 32% of valve prostheses and 64% of the myocardial had positive cultures prior to closure of the wound in a study of 66 patients undergoing open heart surgery.[22] Adhesion mediated infections have proven to be so resistant to both host defenses and medical management with antibiotics that PVE of MHVs, in particular, usually results in the prosthesis having to be removed and replaced.[1] Therefore, it is critical to prevent the infectious organism from adhering to or propagating on the device in the first place.

Some surgeons dip MHVs in antibiotics to lessen the perioperative contamination.[23] However, the drug is rapidly eluted from the prosthesis into the blood and surrounding tissue. Methods to stabilize the antibiotic on prostheses using surfactants also result in relatively fast elution.[24] Other approaches to reducing microbial colonization of medical devices have included coating with drugs to reduce inflammation and surface modification with nonadhesive materials such as silicone, polytetrafluoroethylene, and hydrogels.[25–28] Antibiotic and other drug therapies pose difficulties in dosing appropriately to maximize efficacy and minimize toxicity. Passive coatings aimed at preventing protein and cellular attachment have been shown to be inevitably ineffective.[29]

This study showed that IBAD silver coating for a MHV sewing cuff was tightly adherent and low leaching. Inhibited colonization of the fabric by microorganisms implicated in PVE and may therefore provide added protection from intraoperative contamination, and facilitated controlled tissue ingrowth and incorporation. These data support further investigation into the benefits of providing a MHV prosthesis with an antimicrobial silver modified sewing cuff as an additional option to the cardiac surgeon for the treatment of native valve endocarditis (NVE) or prosthetic valve endocarditis (PVE).

Acknowledgment

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References

1. Lytle BW, Priest BP, Taylor PC, *et al.*: Surgery for acquired heart disease. Surgical treatment of prosthetic valve endocarditis. *Thorac Cardiovasc Surg* 111: 198–210, 1996.
2. Congeni LLD, Addonizio VP, Hopeman AR, Hudson AH: A practical approach to prosthetic valve endocarditis. *Ann Thorac Surg* 43: 460–467, 1987.
3. Edmunds LH: Thrombotic and bleeding complications of prosthetic heart valves. *Ann Thorac Surg* 44: 430–445, 1987.
4. Paschal S, di Summa M, Pisano F, Formosa M, Serra M, Dominioni L: Risk of recurrence after reoperation for prosthetic valve endocarditis. *J Heart Valve Dis* 6: 84–87, 1997.
5. Parnell G, Polesio G: Tentative reference values for gold, silver, and platinum: literature data analysis. *Sci Total Environ* 120: 93–96, 1992.
6. Fox CL, Modak SM: Mechanism of silver sulfadiazine action in burn wound infections. *Antimicrob Agents Chemother* 5: 582–588, 1974.
7. Jansen B, Rinck M, Wolbring P, Strohmaier A, Jähne T: In vitro evaluation of the antimicrobial efficacy and biocompatibility of a silver-coated central venous catheter. *J Biomater Appl* 12: 23–70, 1998.
8. Lindberg M, Lundeberg T: Assessment of silver-coated urinary catheter toxicity by cell culture. *Urol Res* 17: 359–360, 1989.
9. Grimes DL, Morgan HW, Butcher HR, Bellinger WP: The antibacterial effect of treating suture with silver. *Surgery* 73: 122–127, 1973.
10. Kapp M, Steele NS, Hampton T, Pertsemlidis B, Wolfe DE, Okada T: Cytotoxicity of amalgams, alloys, and their elements and phases. *Dent Mater* 7: 68–72, 1991.
11. Klonaris EV, Blendick DF, Stelbrink GA, Kelly RM, Towne JB: Antibiotic-bonded PTFE vascular grafts: the effect of silver acetate on bioactivity following implantation. *J Surg Res* 30: 430–435, 1981.
12. Carnes M, Tobin EJ, Stoddard P, Mesa A, Rizzo L: Bactericidal/static of silver ion-implanted pin surfaces for external fixation: in vitro preliminary observations. Fifth World Biomater Congress Trans, p. 167, 1996.
13. Thomas WA: Relation of the equilibrium contact angle to liquid and constituent. *Adv Chem Ser* 43: 1–51, 1970.
14. Kaelble DH: Dispersion-polar surface tension properties of organic solids. *J Adhesion* 2: 66–81, 1970.
15. Ivarsson BJC, Lerch R, Lang G, *et al.*: Long-term evaluation of prosthetic mitral valves: a phase-/ *Thorac Surg* 46: 173–182, 1988.
16. Wan AT, Conyers RAJ, Coombs CJ, Masterton JP: Determination of silver in blood, urine, and tissues of volunteers and burn patients. *Clin Chem* 37: 1683–1687, 1991.
17. Riley DK, Classen DC, Stevens LE, Burke JP: A large randomized clinical trial of a silver-impregnated urinary catheter. *Am J Med* 98: 349–356, 1995.
18. Grier N: Silver and its compounds, in Block SS (ed), *Disinfection, Sterilization, and Preservation*, Philadelphia, Lea & Febiger, 1977, pp. 333–407.
19. Peterson RC: Pharmacology and toxicology of heavy metals: silver. *Pharmacol Ther [A]* 1: 127–130, 1976.
20. Bambauer R, Mendel P, Schiel R, Stoffregen H: New surface-treatment technologies for catheters used for extracorporeal blood flushing methods. *Biomater Transplant* 24: 226–237, 1995.
21. Williams RL, Doherty PJ, Vince DG, Crawhoff GJ, Williams DF: The biocompatibility of silver. *CRC* 3: 221–243, 1989.
22. Kluge R
tamic
1974
23. Troiski
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1983
24. Farber
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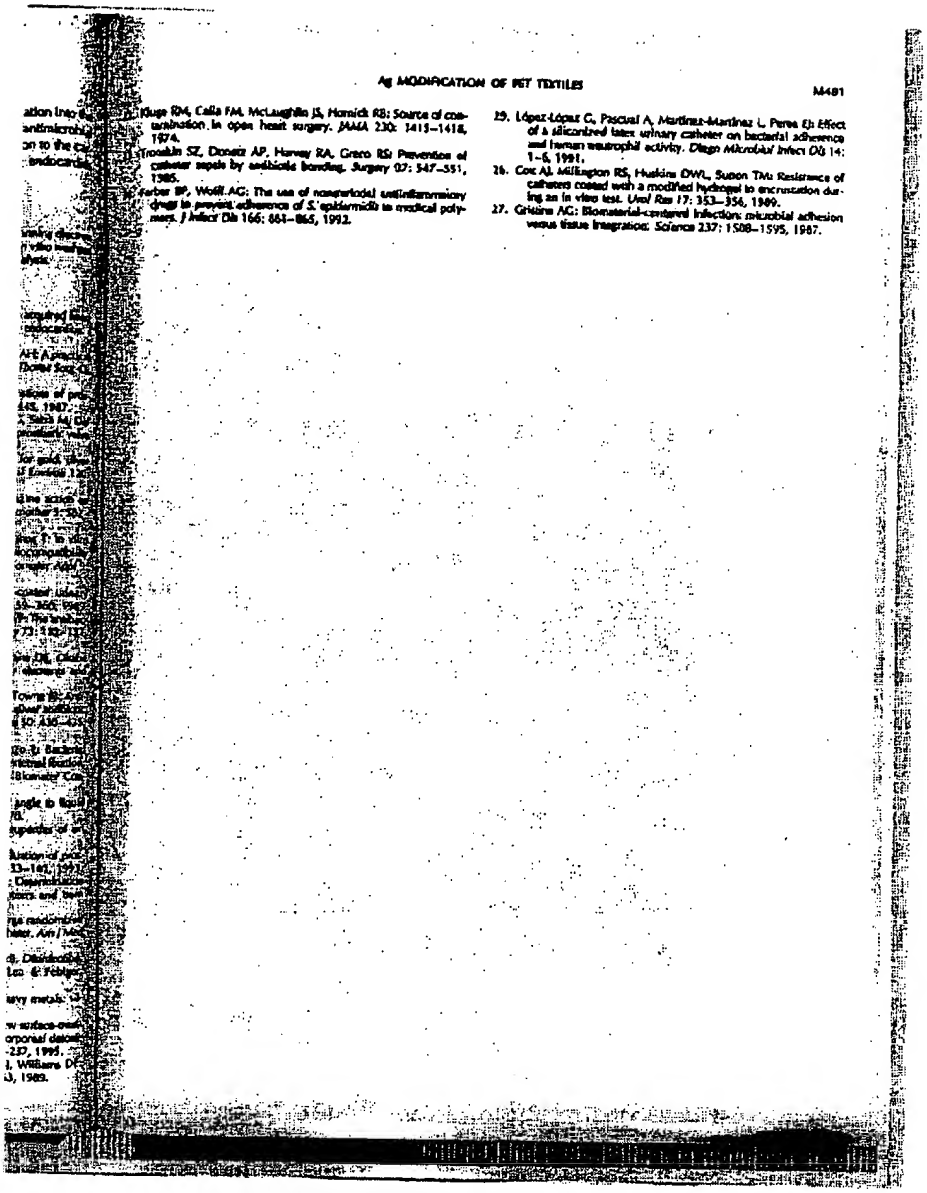

...Rupp RM, Calia FM, McLaughlin JS, Hamrick RB: Source of contamination in open heart surgery. *JAMA* 230: 1415–1418, 1974.

...Trooskin SZ, Donesz AP, Harvey RA, Greco RS: Prevention of catheter sepsis by antibiotic bonding. *Surgery* 97: 547–551, 1985.

...Farber BF, Wolff AG: The use of nonantibiotic antiinflammatory drugs to prevent adherence of *S. epidermidis* to medical polymers. *J Infect Dis* 166: 861–865, 1992.

19. López-López C, Pascual A, Martínez-Martínez L, Perea EJ: Effect of a silicone-coated latex urinary catheter on bacterial adherence and human neutrophil activity. *Diagn Microbiol Infect Dis* 14: 1–6, 1991.

26. Cox AJ, Millington RS, Huskins DWL, Sutton TM: Resistance of catheters coated with a modified hydrogel to encrustation during an in vitro test. *Urol Res* 17: 353–356, 1989.

27. Gristina AG: Biomaterial-centered infection: microbial adhesion versus tissue integration. *Science* 237: 1588–1595, 1987.

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New Polymeric Biocides: Synthesis and Antibacterial Activities of Polycations with Pendant Biguanide Groups

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Acrylate monomers with pendant biguanide groups were successfully synthesized, and their homopolymers and copolymers were prepared with acrylamide. These cationic disinfectants of polymeric forms exhibited high antibacterial activity against gram-positive bacteria, whereas they were less active against gram-negative bacteria. It was found that the activity of the polymeric disinfectants was much higher than that of the monomeric species, and the difference in activity between the polymers and the monomers was discussed on the basis of their contributions to each elementary process of the lethal action.

Quarternary ammonium salts and biguanides, both of which are positively charged at physiological pH, have been used widely as effective antibacterial agents. Their common features are the presence of a positively charged part and a fairly lipophilic part in the same molecule (12). Since the early work of Rose and co-workers (4, 13), biguanides have been employed widely as antimicrobial agents. Currently, chlorhexidine is one of the most popular disinfectants because of its broad spectrum of antibacterial activity, high kill rate, and nontoxicity toward mammalian cells (7).

At the present stage of study, the sequence of elementary events in the lethal action of the cationic disinfectants may be summarized as follows (7): (i) adsorption onto the bacterial cell surface; (ii) diffusion through the cell wall; (iii) binding to the cytoplasmic membrane; (iv) disruption of the cytoplasmic membrane; (v) release of K^+ ions and other cytoplasmic constituents; and (vi) precipitation of cell contents and the death of the cell. Electrophoretic measurements clearly demonstrate that the bacterial cell surface is usually negatively charged. The adsorption of polycations onto the negatively charged cell surface is expected to take place to a greater extent than that of monomeric cations because of the much higher charge density carried by the polycations. Furthermore, binding to the cytoplasmic membrane is also expected to be facilitated by the polycations, compared with that by the monomeric cations, because of the presence of a large number of negatively charged species (such as acidic phospholipids and some membrane proteins) in the membrane (7). Thus, the disruption of the membrane and the subsequent leakage of K^+ ions and other cytoplasmic constituents would be enhanced by the polycations. These considerations would lead to the expectation that cationic disinfectants of polymeric forms exhibit higher antibacterial activity than those of monomeric or dimeric forms.

To examine the advantage of the cationic disinfectants of polymeric forms in antibacterial activity, we prepared ethylenic biguanide derivatives which were either homopolymerizable or copolymerizable to high-molecular-weight polymers. We chose biguanide compounds because of their broad spectrum of activity and nontoxicity as mentioned above. These polymers were found to exhibit higher antibacterial activity than the relevant monomeric species, which would enable us to look into polymer effects in bactericidal action with reference to polymer structure and molecular weight.

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MATERIALS AND METHODS

Preparation. The synthetic route for monomers with pendant biguanide groups is shown in Fig. 1.

4-(2-Hydroxyethyl)aniline hydrochloride(II). p-Aminophenethylalcohol (Tokyo Kasei) (100 g; 0.73 mol) was dissolved in dioxane (1,000 ml), and after filtering, dry hydrochloric acid gas was passed through the solution with vigorous stirring at room temperature for 1 h. The precipitated salt was collected and dried under vacuum (crude product, 120 g; yield, 95%). It was then recrystallized from 2-propanol. Melting point (mp), 170 to 172°C; nuclear magnetic resonance (NMR) (CD_3OD, δ), 2.83 (2H, t, $-CH_2-$), 3.80 (2H, t, $-CH_2OH$), 5.30 (s, broad, NH_3-), 7.40 (4H, s, aromatic).

4-(2-Hydroxyethyl)phenyldicyandiamide(III). The procedure reported by Curd et al. was followed (3). A mixture of II (80 g; 0.46 mol), sodium dicyanamide (Kanto Chemical; 90% pure; 45.6 g), and distilled water (920 ml) was stirred at 90°C for 2.5 h. After cooling to room temperature, the precipitate was collected and dissolved in 2 N sodium hydroxide (100 ml) at 60°C. The insoluble part was removed by filtration, and the filtrate was made acidic (pH 2) with hydrochloric acid. The pale cream precipitate was collected, washed with water, dried under vacuum (64 g; yield, 68%) and then recrystallized from 2-propanol: mp, 172 to 174°C; NMR ($DMSO-d_6$, δ), 2.70 (2H, t, $-CH_2-$), 3.67 (2H, t, $-CH_2OH$), 6.92 (2H, s, dicyandiamide), 7.25 (4H, s, aromatic), 9.02 (1H, s, dicyandiamide). Elemental analysis: Calculated: C, 58.80; H, 5.92; N, 27.44. Found: C, 58.78; H, 5.98; N, 27.45.

4-(2-Acryloyloxyethyl)phenyldicyandiamide(IV). A solution of III (15 g; 0.074 mol) dissolved in tetrahydrofuran-water (10:1 [vol/vol]; 99 ml) was cooled in an ice bath; to this solution acryloyl chloride (Tokyo Kasei; 45 ml; 0.55 mol) was added dropwise with stirring over a period of 2 h, and the reaction mixture was left overnight at room temperature. It was then poured into a large excess of water, and the precipitate was collected and dried under vacuum (18 g; yield, 95%). It was recrystallized from an acetone-benzene (1:2 [vol/vol]) mixture: mp, 157 to 159°C; NMR ($DMSO-d_6$, δ), 2.90 (2H, t, $-CH_2-$), 4.30 (2H, t, $-CH_2O-$), 5.9-6.4 (3H, m, vinyl), 6.95 (2H, s, dicyandiamide), 7.30 (4H, s, aromatic), 9.05 (1H, s, dicyandiamide). Elemental analysis: Calculated: C, 60.45; H, 5.46; N, 21.69. Found: C, 60.10; H, 5.21; N, 21.52.

N^1-4-(2-Acryloyloxyethyl)phenyl-N^5-4-chlorophenylbiguanide hydrochloride(VI). 4-Chloroaniline hydrochloride (5.5 g;

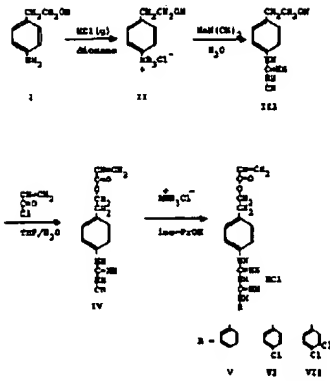

FIG. 1. Synthetic route for monomers with pendant biguanide groups.

0.034 mol), IV (8.7 g; 0.034 mol), and 2-propanol (30 ml) were refluxed in the presence of a small amount of hydroquinone for 15 min. The precipitate which formed on cooling was collected, washed with 2-propanol, dried under vacuum (9.7 g; yield, 69%), and recrystallized from 2-propanol: mp, 204 to 206°C; NMR (CD_3OD, δ), 2.93 (2H, t, $-CH_2-$), 4.33 (2H, t, $-CH_2O-$), 4.77 (6H, s, biguanide), 5.8-6.4 (3H, m, vinyl), 7.3 (4H, s, aromatic), 7.35 (4H, s, aromatic); (DMSO-d_6, δ), 7.0-7.6 (broad, biguanide). Elemental analysis: Calculated: C, 54.04; H, 5.01; N, 16.58; Cl, 16.79. Found: C, 54.14; H, 4.81; N, 16.15; Cl, 16.13. N^1-4-(2-Acryloyloxyethyl)phenyl-N^5-phenylbiguanide hydrochloride(V) and N^1-4-(2-acryloyloxyethyl)phenyl-N^5-3,4-dichlorophenylbiguanide hydrochloride(VII) were prepared similarly (yield, 49% for V and 45% for VII; their structures were confirmed by NMR).

Polymerization. Homopolymers of V, VI, and VII. Polymerization was carried out at 60°C in dimethylformamide (DMF) with 2,2'-azobis(2-amidinopropane)dihydrochloride (Wako Chemical) as an initiator. Each polymerization tube was charged with desired amounts of the monomer, the initiator, and DMF (30 ml) (see Table 1). It was then degassed by three freeze-pump-thaw cycles under high vacuum, sealed off, and placed in a constant temperature bath at 60°C. After the period indicated in Table 1, the polymerization tube was opened, and the content was poured into an excess of acetone (300 ml). The precipitated polymer was filtered off, washed with acetone, and dried under vacuum. The conversion for each polymer is shown in the fifth column of the table. Each polymer was purified by reprecipitation of the methanol solution into a large excess of acetone. M_w was determined in methanol and listed in the last column of the table. It is evident that the values of M_w are not so different among the three polymers.

TABLE 2. Copolymerization of VI with acrylamide[a]

Copolymer	Mol fraction of VI in:		Conversion (%)	M_w[b]
	Monomer	Polymers		
VIII	0.200	0.352	92	—[c]
IX	0.400	0.563	90	18,900
X	0.598	0.426	83	34,500
XI	0.793	0.506	83	31,500

[a] Solvent, DMF-H_2O (1:1 [vol/vol]); initiator, 2,2'-azobis-2-amidinopropane · 2 HCl; temperature, 60°C; time, 9 h.
[b] Determined with a low-angle, laser light-scattering photometer (KMX-6) in methanol.
[c] —, Not soluble in methanol.

Copolymers of VI with acrylamide. Copolymerization was conducted at 60°C in mixed solvent of DMF-water (1:1 [vol/vol]) with the same initiator as that used in the homopolymerization. Each polymerization vessel was charged with the predetermined amounts of VI and acrylamide, whereas the total weight of the monomers was kept constant (100 g/liter). The concentration of the initiator was 4.0 g/liter. The polymerization procedure was the same as that of homopolymerization. The copolymers obtained were highly hygroscopic, so that care was taken not to leave them in air for a long period, particularly when filtered. The conversion for each copolymer is listed in Table 2. The copolymer composition was determined on the basis of the absorption at 257 nm due to the aromatic biguanide hydrochloride. To obtain the value of ε for N^1-4-alkylphenyl-N^5-4-chlorophenylbiguanide hydrochloride, N^1-4-(2-hydroxyethyl)-N^5-4-chlorophenylbiguanide hydrochloride was used as model compound: λ_{max} = 230 nm ($\varepsilon = 1.83 \times 10^4$); 257 nm ($\varepsilon = 1.74 \times 10^4$) in water. The molecular weight is listed in the last column of Table 2.

Antibacterial assessment. Freeze-dried ampules of *Staphylococcus aureus* (IFO 12732) and *Escherichia coli* (IFO 3806) were opened, and a loopful of each culture was spread to give single colonies on nutrient agar and incubated at 37°C for 24 h. A representative colony was picked off with a wire loop and placed in 10 ml of nutrient broth (peptone [Wako Chemical], 10 g; NaCl, 5.0 g; beef extract [Wako Chemical], 5.0 g in 1,000 ml of sterile distilled water [pH 6.8]), which

TABLE 1. Homopolymerization of the biguanide monomers[a]

Monomer	Monomer concentration (g/liter)	Initiator concentration (g/liter)	Polymerization time (h)	Conversion (%)	M_w[b]
V	100	1.14	6.5	70	11,700
VI	139	0.83	6.5	81	11,900
VII	97	1.18	6.0	57	12,100

[a] Solvent, DMF; initiator, 2,2'-azobis-2-amidinopropane · 2 HCl; temperature, 60°C.
[b] Determined with a low-angle, laser light-scattering photometer (KMX-6) in methanol.

TABLE 3. Antibacterial activity of biguanide compounds[a]

Compound	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Aerobacter aerogenes*	*Pseudomonas aeruginosa*
VI	10–33	33–66	66–100	100–330	100–330
Poly V	100–330	100–330	>1,000	>1,000	>1,000
Poly VI	100–330	100–330	660–1,000	660–1,000	>1,000
Poly VII	100–330	100–330	660–1,000	660–1,000	660–1,000
XII	100–330	100–330	100–330	660–1,000	660–1,000

[a] MIC (μg/ml) determined by the spread plate method.

was then incubated overnight at 37°C. At this stage, the culture of *S. aureus* contained ~10^8 cells per ml, and that of *E. coli* contained ~10^9 cells per ml. By diluting with sterile distilled water, culture containing ~10^6 cells per ml was prepared for each strain which was used for antibacterial test. Since the biocides were not completely soluble in water as 1% concentrate, they were dissolved in methanol-water (3:9 [vol/vol]) at first and then diluted with sterile distilled water so as to give the correct final concentration when 18.0 ml of the biocide solution was combined with 2.0 ml of the bacterial culture. It was confirmed that methanol used for the preparation of 1% concentrate did not affect the result of the antibacterial tests. Exposure of bacterial cells to the biocide was started when 2.0 ml of the bacterial culture containing ~10^6 cells per ml was added to 18.0 ml of the biocide solution which was preequilibrated at 37°C. At the same time, 2.0 ml of the same culture was added to 18.0 ml of saline, decimal dilutions were prepared, and the starting cell concentration was enumerated by the spread plate method. At various contact times 1.0-ml portions were removed and quickly mixed with 9.0 ml of neutralizer solution (20% Tween 80 plus 3% azolectin in nutrient broth), and then decimal serial dilutions were prepared from this by taking 0.2 ml into 1.8 ml of saline and mixing. From these dilutions the surviving bacteria were counted by the spread plate method. After inoculation, the plates were incubated at 37°C, and the colonies were counted after 48 h. The counting was done in triplicate every time.

Conventional antimicrobial susceptibility testing by the spread plate method was conducted as described previously (8).

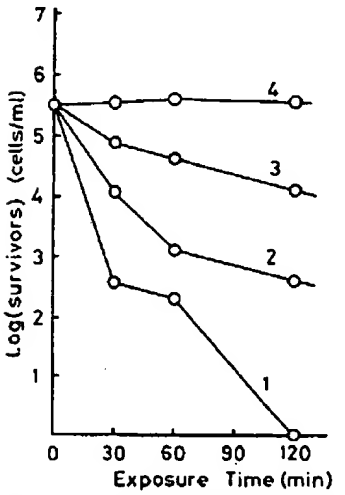

FIG. 2. Log (survivors) versus exposure time plots for the homopolymers against *S. aureus* were as follows: 1, poly V; 2, poly VI; 3, poly VII; and 4, control. Concentration, 1.2 μM, based on the monomer unit (0.5 μg/ml for poly VI).

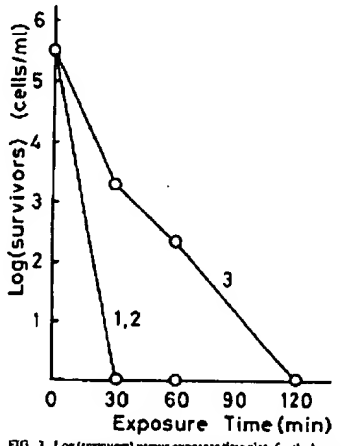

FIG. 3. Log (survivors) versus exposure time plots for the homopolymers against *S. aureus* were as follows: 1, poly V; 2, poly VI; and 3, poly VII. Concentration, 2.4 μM, based on the monomer unit (1.0 μg/ml for poly VI).

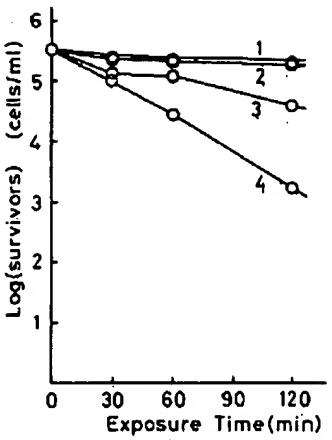

FIG. 4. Log (survivors) versus exposure time plots for the monomer VI against *S. aureus* at the following concentrations: 1, 1.2 μM; 2, 12 μM; 3, 120 μM; and 4, 237 μM.

Measurements. The M_w of polymers was determined with a KMX-6 low-angle, laser light-scattering photometer. The absorption spectra were recorded with a Shimazu UV-200 spectrometer, and ^{1}H-NMR spectra were recorded on a JEOL JNM-PM 60.

RESULTS AND DISCUSSION

Many trials have been done to prepare acrylate monomers by esterification of N^1-4-(2-hydroxyethyl)phenyl-N^5-4-chlorophenylbiguanide hydrochloride(XII). The latter compound was prepared by refluxing an equimolar mixture of III and 4-chloroaniline hydrochloride in 2-ethoxyethanol for 15 min (yield, 65%). The Schotten-Baumann reaction between XII and acryloyl chloride in pyridine was not successful, giving apparently a triazine derivative with no vinyl group (by NMR), as the conversion of biguanides into guanamines seemed to take place in the presence of acylating agents under basic conditions (11). The same reaction in different solvents (DMF and water) also was not successful, though in DMF a very small amount of the acrylate monomer VI was produced. Esterification of XII by the use of dicyclohexylcarbodiimide and acrylic acid in DMF and pyridine was tried and found not to proceed. Then the strategy in synthetic route was changed: first, dicyandiamide with vinyl group IV was prepared, and then the step for biguanide formation was followed. By this procedure, the acrylate monomers with a pendant biguanide group were successfully prepared despite the fact that biguanides are highly susceptible to condensation with esters to give triazines (11).

Table 3 shows MICs evaluated by the conventional spread plate method. The two figures for each strain indicate the range of MIC; growth of the bacterium could be seen as visual colonies below the lower concentration limit of MIC, whereas no colonies were observed above the higher limit. Consequently, the exact MIC is supposed to lie between these two values. A general trend can be seen from the table that the biguanide monomers and polymers are active against gram-positive bacteria, whereas they are less active against gram-negative bacteria. It is also evident that XII, which has a free hydroxy group, is less active than VI in which the hydroxy group is esterified. Another characteristic seen in the table is that the monomer VI is more active than the polymers.

Here we reached the conclusion that the method of evaluating antibacterial activity of biguanide compounds must be reexamined. The polymeric biguanides are polycations and have a strong tendency to interact with some constituents of media used to cultivate bacteria. They interact strongly with negatively charged species (such as sodium caseinate) and produce insoluble complexes. This complexation may lead to inactivation of the polymeric biguanides when their activity was evaluated on the growth media. To eliminate the interference by the constituents in the growth media, the bactericidal assessment was performed in sterile water.

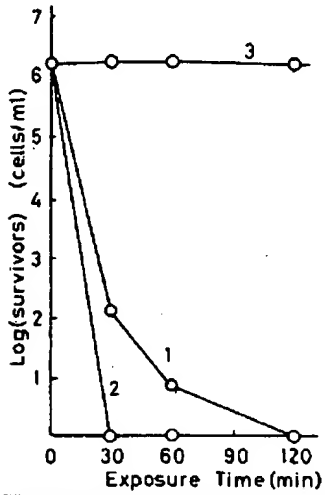

FIG. 5. Log (survivors) versus exposure time plots for VI and poly VI against *E. coli* were as follows: 1, VI; 2, poly VI; and 3, control. Concentration, 25 μM.

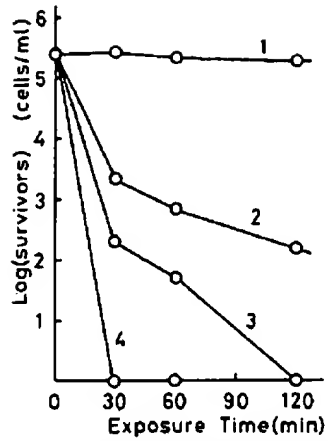

FIG. 6. Log (survivors) versus exposure time plots for the copolymer XI against *S. aureus* at the following concentrations: 1, 0.2 μM; 2, 1.2 μM; 3, 2.4 μM; and 4, 12 μM. The concentrations were calculated on the basis of the biguanide monomer units.

Figure 2 shows log (survivors) versus exposure time plots for the homopolymers of V, VI, and VII (poly V, poly VI, and poly VII, respectively) against *S. aureus*. Exposure of the polymers to bacterial cells was carried out in sterile water. The concentration of the polymers was 1.2 μM, based on the monomer unit (0.5 μg/ml for poly VI). Poly V was the most active among the three. The difference in activity among the three polymers is not well understood at the moment. Figure 3 shows the log (survivors) versus exposure time plots for the homopolymers against *S. aureus* at 2.4 μM (1.0 μg/ml for poly VI). All of the bacterial cells were killed within 30 min when exposed to poly V and poly VI. At the concentrations higher than 12 μM, all of the bacterial cells were killed within 30 min when exposed to any of the homopolymers. Figure 4 indicates the same plots for the monomer VI. Exposure of the bacterial cells to the monomer VI at the concentration 50 times as high as those of the polymers (120 μM, 50 μg/ml) exerted little effect on reducing the number of survivors (Fig. 4, curve 3). From Fig. 2 through 4, it is evident that the polymers (poly V, poly VI, and poly VII) are much more active than the monomer VI when exposed to the bacterial cells without interfering materials.

A similar result was obtained against gram-negative strain *E. coli*. Figure 5 shows a comparison in bactericidal activity between VI and poly VI at the concentration of 95 μM (40 μg/ml). It is clear that poly VI is more active than VI in this case as well.

Figure 6 shows the log (survivors) versus exposure time plots for XI, which contains 50.6 mol% of biguanide monomer units in the copolymer against *S. aureus*. The concentrations shown in Fig. 6 were those calculated on the basis of the biguanide monomer units. Copolymers with different biguanide compositions exhibited a similar concentration dependence on bactericidal activity.

The most probable explanation for the higher activity of the polymers may be given by considering their contribution to each elementary process in the lethal action (see above). The bacterial cell surface is negatively charged as evidenced by electrophoretic mobility. Absorption of polycations onto the negatively charged cell surface is supposed to be much more favored than that of monomeric cations on account of the higher charge density of the polyelectrolytes. Thus, process i is expected to be more enhanced for polycations compared with that for monomeric cations.

With respect to process ii, the polycations have a disadvantage. The gram-positive strains have a rather simple cell wall composed of a rigid peptidoglycan layer which allows foreign molecules to come inside without much difficulty (2). Thus, polycations with relatively low molecular weights as used in this study might diffuse easily through the cell walls of gram-positive bacteria. On the other hand, in the case of gram-negative bacteria, there is another bilayer membrane outside the peptidoglycan layer (outer membrane). Because of the outer membrane, foreign molecules are not capable of diffusing easily through the cell wall (2). This could be a disadvantage to the polymeric biocides, since they have larger molecular size than the monomeric ones. However, as is seen in Fig. 5, poly VI with M_w of 11,900 exhibited higher activity against *E. coli* than VI, which might suggest that this type of polycation can reach the cytoplasmic membrane of the gram-negative species after the partial breaking down of the outer membrane.

It is still ambiguous how the biguanides as well as other cationic disinfectants interact with the cytoplasmic membrane with subsequent disruption, although it has been reported that they interact strongly with negatively charged species present in the membrane such as acidic phospholipids and some membrane proteins (1, 6, 9, 10, 14). Binding of the polymeric biguanides to the cytoplasmic membrane (process iv) is supposed to take place more preferably than that of the monomeric ones due to stronger interaction of the former with negatively charged species present in the membrane. This would result in faster disruption of the membrane (process iii) by the polymeric biocides with subsequent release of the cytoplasmic constituents (process v), followed by the death of the cells (process vi). Although the polymeric biocides have a disadvantage in diffusing through the cell walls, overall activity of the polymeric biocides would, after all, be higher than that of the monomers.

In conclusion, the evaluation of antibacterial activity of the polymeric biguanides is complicated by the fact that they interact with some constituents of culture media, and because of this incompatibility evaluation of bacteriostatic activity on growth media will not be precise. In a clean system in which there are no interfering materials such as negatively charged macromolecules, the polymeric biguanides are much more active than the monomeric species. The higher activity of the polymers may be accounted for by their stronger interactions with the cell surface and the cytoplasmic membrane of bacteria as the primary process of the lethal action.

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LITERATURE CITED

1. Bernard, E., J. F. Faucon, and J. Dufourcq. 1982. Phase separations induced by melittin in negatively-charged phospholipid bilayers as detected by fluorescence polarization and differential scanning calorimetry. Biochim. Biophys. Acta 688:153–162.
2. Costerton, J. W., and K.-J. Cheng. 1975. The role of the bacterial cell envelope in antibiotic resistance. J. Antimicrob. Chemother. 1:363–377.
3. Curd, F. H. S., J. A. Hendry, T. S. Kenny, A. G. Mowry, and F. L. Rose. 1948. Synthetic antimalarials. XXVIII. An alternative route to N^1-aryl-N^5-alkyldiguanides. J. Chem. Soc. 1630–1636.
4. Curd, F. H. S., and F. L. Rose. 1946. Synthetic antimalarials. X. Some aryl-diguanide derivatives. J. Chem. Soc. 729–737.
5. Davies, A., M. Bentley, and B. S. Field. 1968. Comparison of the action of vanocil, cetrimide and chlorhexidine on *Escherichia coli* and its spheroplasts and the protoplasts of Gram negative bacteria. J. Appl. Bacteriol. 31:448–461.
6. El Mashak, E. M., and J. F. Tocanne. 1980. Polymyxin B-phosphatidylglycerol interactions. A monolayer (π, ΔV) study. Biochim. Biophys. Acta 596:165–179.
7. Franklin, T. J., and G. A. Snow (ed.). 1981. Antiseptics, antibiotics and the cell membrane, p. 58–78. *In* Biochemistry of antimicrobial action. Chapman and Hall, London.
8. Ike, Y. 1980. Kinsen heiban kiban'ho, p. 74–82. *In* S. Mitsuhashi (ed.), Yakuzai kanjussi sokuteiho (Antimicrobial susceptibility testings). Kodansha, Tokyo.
9. Ikeda, T., A. Ledwith, C. H. Bamford, and R. A. Hann. 1984. Interaction of a polymeric biguanide biocide with phospholipid membranes. Biochem. Biophys. Acta 769:57–66.
10. Ikeda, T., S. Tanaka, and M. Watanabe. 1983. Interaction of biologically active molecules with phospholipid membranes. 1. Fluorescence depolarization studies on the effect of polymeric biocide bearing biguanide groups in the main chain. Biochim. Biophys. Acta 735:380–386.
11. Kaiser, F., and E. D. Pitchforth. 1968. The chemistry of biguanides. Fortschr. Chem. Forsch. 10:375–472.
12. Lynn, B. 1980. Chemical disinfectants, antiseptics and preservatives, p. 155–184. *In* W. B. Hugo and A. D. Russell (ed.), Pharmaceutical microbiology. Blackwell Scientific Publications, Oxford.
13. Rose, F. L., and G. Swain. 1956. Bisbiguanides having antibacterial activity. J. Chem. Soc. 4422–4425.
14. Shai, Y., and H.-J. Gallo. 1981. Polymyxin interaction with negatively charged lipid bilayer membranes and the competitive effect of Ca^{2+}. Biochim. Biophys. Acta 649:626–633.

value (13.8 ea.) computed on the basis of molar refractions[1] to within experimental error. The relatively high loss of the atactic material is attributed to dipolar impurities which are probably also present in the isotactic material. (In the latter case they are probably too rigidly held in the crystallites to rotate.) The only dipolar group which is consistent with the infrared spectra is the double bond, which is only weakly dipolar, i.e., ~0.4 Debye. If one assumes that the loss of the atactic material is due entirely to the unsaturation, the Sillars[2] relation indicates (as a lower limit) that about 2% of the C—C bonds are unsaturated. Infrared and chemical evidence indicate double bond concentrations of less than 1%. The observed loss is probably due to the presence of several polar species, each in low concentration.

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References

1. C. F. Prutton, and S. H. Maron, *Fundamental Principles of Physical Chemistry*, Macmillan, New York, 1950.
2. R. W. Sillars, *Proc. Roy. Soc.*, A169, 66 (1938–39).

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A New Method for the Preparation of Graft Copolymers. Polymerization Initiated by Ceric Ion Redox Systems

We have found that certain ceric salts, such as the nitrate and sulfate, form very effective redox systems in the presence of organic reducing agents such as alcohols, thiols, glycols, aldehydes, and amines. The oxidation-reduction produces cerous ions and transient free radical species capable of initiating vinyl polymerization.

Duke and co-workers[1,2] have shown that ceric salts form complexes with alcohols and glycols, and that the disproportionation of these complexes is the rate determining step of the oxidation-reduction. In the case of alcohols, the mechanism of the initiation reaction can be written quite generally as follows:

$$Ce^{IV} + RCH_2OH \rightleftharpoons B \xrightarrow{K_d} Ce^{III} + H^+ + R\dot{C}HOH \text{ (or } R\dot{C}H_2O\text{-)}$$

where Ce^{IV} represents the ceric complexes as they exist in aqueous solution, B the ceric-alcohol complex, and $R\dot{C}HOH$ a free radical. If a vinyl monomer is present, the free radical initiates polymerization.

The most important feature of the oxidation with ceric ion is that it proceeds via a single electron transfer with the formation of free radicals on the reducing agent. Thus, if the reducing agent is a polymeric molecule such as polyvinyl alcohol or cellulose, and the oxidation is carried out in the presence of a vinyl monomer, the free radical produced on the polymeric molecule (backbone) initiates polymerization to produce a graft copolymer. This method of grafting yields substantially pure graft copolymers since the free radicals are formed exclusively on the backbone.

Although, in the absence of a reducing agent, ceric salts do initiate the polymerization of acrylonitrile,[1] the data of Table I show that with ceric nitrate a long induction period is obtained. In the case of acrylamide, although no induction period is observed, polymerization proceeds at a very low rate.

TABLE I
Effect of Reducing Agent on Initiation by Ceric Ion
(Ceric ammonium nitrate concentration = 2.5×10^{-3} mole/l.; hydrogen ion concentration = 2.5×10^{-1} mole/l.)

Polyvinyl alcohol, base mole/l.	Monomer mole/l.	Temperature, °C.	Initial rate, %/min.
0.9	0.75 acrylonitrile	0	1.34[a]
None	0.75 acrylonitrile	0	[b]
0.22	0.70 acrylamide	20	3.00[a]
None	0.70 acrylamide	30	0.04[a]

[a] No induction period
[b] No polymer after 1 hour.

A typical graft copolymer of polyacrylamide on polyvinyl alcohol was prepared as follows: 2.5 ml. of a 0.1 *M* solution of ceric ammonium nitrate in 1 *M* nitric acid was added to a solution of 5 g. acrylamide and 1 g. polyvinyl alcohol (Elvanol 70-05) in 97.5 ml. water. Polymerization was carried out in an atmosphere of nitrogen at 20°C. After 1 hour, the solution was poured into an excess of acetone to precipitate the gross polymer. The conversion of acrylamide was 93%. Fractional precipitation of the gross polymer showed that no free polyacrylamide was present.

A number of graft copolymers have been studied in some detail. For example, acrylamide, acrylonitrile, and methyl acrylate were grafted onto low molecular weight polyvinyl alcohol in the presence of ceric ammonium nitrate. The graft copolymers obtained were all soluble in suitable solvents, indicating that practically no crosslinking had taken place. The preparation of these copolymers and the determination of the grafting efficiencies will be reported later. Our data show that the ceric ion technique permits high-efficiency grafting on a large variety of polymeric backbones, both natural and synthetic. Among the effective backbones are cellulose and other polyglucosides, such as dextrans and starches, and polygalactosides, such as the carrageenans. A much larger variety is available from synthetic polymers, since these can be tailored to include a reactive group. For example, a small portion of acrolein can be copolymerized with various monomers to give reactive backbones having widely differing properties. It is not necessary for the backbone polymers to be water soluble; they can be prepared in latex form and grafting carried out in emulsion.

References

(1) F. R. Duke and A. A. Forist, *J. Am. Chem. Soc.*, 71, 2790 (1949).
(2) F. R. Duke and R. F. Bremer, *J. Am. Chem. Soc.*, 73, 5179 (1951).
(3) J. Saldick, *J. Polymer Sci.*, 19, 73 (1956).

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J. MACROMOL. SCI.–CHEM., A22(5-7), pp. 579–590 (1985)

The Preparation and Properties of Acrylic and Methacrylic Acid Grafted Cellulose Prepared by Ceric Ion Initiation. Part I. Preparation of the Grafted Cellulose

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ABSTRACT

The ceric ion method has been used to graft acrylic acid directly onto cellulose with a minimum amount of homopolymer. The method utilizes the pretreatment of the cellulose with ceric ammonium nitrate followed by washing out any excess of the catalyst. Oxygen can be present with the pretreatment step, but must be excluded during the grafting reaction itself. The process, which is entirely aqueous in nature, would appear to be quite practical on a large scale. Wet strengthened papers which are essential for the use of the grafted products as ion-exchange media can also be grafted but with adequate but lower yields than with the untreated paper. Apart from ion exchange, the products are of considerable potential value as water sorbing agents. Methacrylic acid gave lower grafting yields than acrylic acid, but these were much improved at higher temperatures.

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INTRODUCTION

There is considerable industrial and academic interest in cellulose-g-poly(acrylic acid) and cellulose-g-poly(methacrylic acid) copolymers. The products are of value because of their ion-exchange properties and, in the form of their alkali metal salts, their high water absorbency. When prepared in a special way, the so-called super-water sorbents can be obtained. These can retain more than 30 times their weight in water and have potential applications for sanitary and medical uses, as soil conditioners, and many other applications. The usual method of preparation has been to graft, using chemical initiation, particularly ceric ion, monomers such as acrylonitrile and/or acrylate and methacrylate esters followed by alkaline hydrolysis. One of the main reasons for this two-step approach has been the large amounts of homopolymer formed when acrylic or methacrylic acids are grafted directly with chemical initiators. Excessive homopolymer can be avoided, however, by using high-energy radiation. The direct, mutual method requires the use of free radical inhibitors in the aqueous phase to reduce homopolymer to a minimum. The preirradiation technique essentially eliminates the problem [1]. Both methods are being pursued industrially to produce acrylic-acid-grafted polyethylene films for battery separators.

There is a growing interest and acceptance of high-energy radiation as a useful industrial initiator for polymerization grafting and crosslinking. In spite of this, many industries prefer the more familiar chemical approaches. In the work previously reported using chemical initiation, the grafting yields were rather low and considerable homopolymer was formed [2-4]. More recently, Gangueux, Watticz, and Marechal [5] have reported a method for grafting acrylic acid to cellulose powder, Solka Floc, for use in textile wastewater treatments. The cellulose was pretreated with ceric ion in aqueous solutions, the excess removed by squeezing and the acrylic acid introduced in benzene solution. Since the hydrated ceric ions could not easily diffuse into the benzene solution of the monomer, comparatively little homopolymer was formed. Grafting yields of up to 70%, based on 100 parts of cellulose, were obtained but still with about 45% homopolymer. Quite recently McDowall, Gupta, and Stannett [6] have further refined the method with yields up to 60% and less than 5% homopolymer. However, this method still used organic solvents and for industrial use, particularly in the pulp and paper industry, all-aqueous systems are greatly preferred. The pretreatment of wood pulp with ceric ammonium nitrate followed by the addition of aqueous acrylic acid solution was found to give up to 18% grafting, together with an unknown amount of homopolymer, in an unpublished report by Ogiwara, Kubota, and Tanizaki [7]. This method has been further explored here in considerable detail and developed into a viable all-aqueous system. It should be pointed out that the use of acrylonitrile presents toxicity problems as it is a known carcinogen. In this respect, the direct grafting of acrylic acid offers a definite advantage in spite of the lower cost of acrylonitrile. This cost advantage is not found, however, when the nontoxic acrylic esters are used. The results of this work are reported in this paper.

EXPERIMENTAL

Materials

Filter paper prepared from bleached sulfite softwood pulp was the cellulose used throughout the investigation.

Ceric ammonium nitrate (CAN), reagent grade, was obtained from the Fisher Scientific Company and used without further purification.

Acrylic acid (AA) and methacrylic acid (MAA) were obtained from the Rohm and Haas Co. and purified by distilling twice under vacuum in the presence of copper powder to prevent polymerization. Other monomers were obtained from the Fisher Scientific Company and distilled at atmospheric pressure before use.

Nitrogen gas containing less than 1 ppm of oxygen was obtained from Air Products Inc.

Grafting Procedure

After considerable experimentation, the following procedure was adopted. A 500-mL glass kettle fitted with an electric stirrer and nitrogen inlet and outlet parts was used. The nitrogen inlet had a fritted glass end. The temperature was controlled at 25°C by use of a thermostatted water bath. The filter paper was separated from the stirrer by a spacer consisting of two glass rings connected by vertical rods.

The actual procedure consisted of the following steps: 1) pretreatment of the paper with catalyst solution, 2) washing out excess catalyst, 3) reaction with the monomer solution, 4) washing out any homopolymer formed. The details of each step were as follows:

1. In all experiments, a 8 in. × 4 in. filter paper (machine direction × cross direction) was used. Fresh catalyst solution was prepared by dissolving a known amount of ceric ammonium nitrate in 0.1 N nitric acid and mixing with deionized water to obtain the required concentration. Typically, 300 mL of the catalyst solution containing 105 mL of 0.1 N nitric acid was used in the pretreatment step. The catalyst solution in the glass vessel was maintained at a constant temperature by immersing it in a constant temperature water bath. The solution was bubbled through a sintered glass with a continuous stream of nitrogen gas. Cellulosic filter paper was placed in the catalyst solution for the required amount of time. The typical pretreatment time

was 2 h. The cerium content of the paper was determined when needed by neutron activation analysis.

2. After pretreatment, the paper was removed from the catalyst solution and rinsed thoroughly with deionized water twice. At least 200 mL of deionized water was used each time. Then the filter paper was passed through rubber press rolls to squeeze out excess water. Thorough washing of the filter paper was found to be necessary in order to prevent homopolymer formation in the reaction stage.

3. The reaction medium in the reaction kettle, consisting of deionized water and nitric acid, was first degassed by passing nitrogen through the solution for at least 30 min. The catalyst-treated cellulosic filter paper was then placed in the reaction kettle to which was added 30 mg of copper powder, to inhibit homopolymer formation, and the monomer(s). Nitrogen gas continued to be bubbled through the medium during the whole reaction period. After the reaction time was completed, the grafted paper was removed from the solution and was washed thoroughly to remove the soluble contents. The typical pretreatment and grafting conditions used are summarized in Table I.

4. Finally, the grafted paper was extracted with the solvent appropriate for the homopolymer, viz., methanol for polyacrylic acid, benzene for polymethyl acrylate, and N,N-dimethylformamide, at 80°C, for polyacrylonitrile. The methanol and benzene extractions were

TABLE I. Typical Reaction Conditions

A. Pretreatment conditions:	
Total volume	300 mL
CAN concentration	200 mmol/L
Nitric acid concentration	0.035 N
Time	2.0 h
Temperature	25°C
B. Grafting conditions:	
Total volume	600 mL
Monomer concentration	5% by volume
Nitric acid concentration	0.27 N
Copper powder	30 mg
Time	5.0 h
Temperature	25°C

carried out in a Soxhlet apparatus for 24 h. The percent graft was calculated by weighing after drying at 40°C under vacuum and based on the original dry weight of the cellulose. This value represents the unextractable polymer in the cellulose substrate. Twenty-four hours was found to lend to constant weights.

RESULTS AND DISCUSSION

Effect of the Processing Variables on Acrylic Acid Grafting

The Pretreatment Step

The cerium content of the filter paper was determined, using neutron activation analysis, after various times of immersion in the CAN solution containing nitric acid. Typical results are shown in Fig. 1 together with the conditions used. Although nitrogen was used, it was found subsequently that air was also suitable for this step of the process (see also Ref. 6). The amount of ceric ion sorbed rose steadily to a maximum at 2 h and then decreased somewhat. The decrease, although reproducible, was somewhat unexpected. It was presumably due to some concurrent decomposition and solution of the ceric-cellulose complex. A detailed study of the sorption of CAN by cellulose together with a discussion of the various reactions and mechanisms has been presented by Ogiwara, Ogiwara, and Kubota [8]. A pretreatment time of 2 h, giving the maximum cerium concentration, was selected for all the experiments reported in this study.

The Grafting Process

Typical grafting-time curves at two monomer concentrations are shown in Fig. 2. The rate increased somewhat with time, perhaps because of oxygen exhaustion or monomer diffusion effects. Good yields were obtained in 2–5 h.

The effect of monomer concentration is presented in Fig. 3. As is normal, except at high concentrations where swelling effects may be involved, the yield steadily increases; at 8% monomer solutions, more than 200% grafting was obtained.

Varying the concentration of CAN in the pretreatment step also affects the yield and the homopolymer formation as shown in Fig. 4. The falling off of yield at higher concentrations is a well-known phenomena [9] and ascribed to the increasing participation of the ceric ion in the termination process. Since this is particularly marked in the homopolymer yield, presumably noncomplexed ceric ions are present, some of which can escape into the supernatant monomer solution. At 15 mmol/L of CAN, there is nearly 45% grafting with essentially no homopolymer. At 20 mmol/L, grafting has increased to 126% but together with 22% of homopolymers.

FIG. 1. The rate of adsorption of cerium by cellulose at 25°C from 20 mmol CAN/L in 0.035 N nitric acid solution.

The effect of pH is quite dramatic both with acrylic acid and acrylonitrile as can be seen in Fig. 5. These effects have been noted also by Terada and Matsuki [10], by Ogiwara et al. [7], and by Kabai et al. [11] in the direct polymerization of acrylonitrile. It is believed that the dissociation constants of the cellulose, or other, ceric ion complexes sharply decrease at higher pH values.

The well-known inhibiting effect of oxygen was also operative in this work. For example, the grafting yield dropped from 125% under nitrogen to only 2% under similar experimental conditions but in air.

FIG. 2. The rate of grafting of acrylic acid to filter paper at 25°C. Pretreated with 20 mmol/L in 0.035 N nitric acid solution for 2 h. Reaction in 0.27 N nitric acid at 8% (○) and 5% (○) aqueous monomer concentrations by volume.

The Grafting of Methacrylic Acid

Methacrylic acid was graft copolymerized onto cellulose under various conditions; the results are presented in Table 2. When graft copolymerization was carried out at a temperature of 25°C, poor graft yields (less than 10% "graft") were obtained even under different concentrations of the catalyst in the pretreatment step, whereas under identical conditions, much higher graft yields (higher than 120% graft) were achieved with acrylic acid (see Fig. 4). Therefore attempts were made to graft methacrylic acid at a higher temperature, i.e., 50°C. It is apparent from the results presented in Table 2 that good graft yields are achievable at a higher temperature. Similarly, in the graft copolymerization of methacrylic acid by the conventional

FIG. 3. Effect of monomer concentration on percent grafting at 25°C for 5 h in 0.27 N nitric acid solution. Pretreated as in Fig. 2.

ceric ion process, Ogiwara et al. [7] also obtained very poor graft yields at low temperature (0°C) but higher grafting at 45 and 60°C. Zahran et al. [1] found higher grafting yields both to rayon and cotton using the preirradiation technique but at 70°C. It is clear that considerable further work is needed with the two monomers before a reasonable explanation can be presented.

Grafting of Acrylic Acid to Wet Strength Resin Treated Papers

The polyacrylic acid grafted papers possessing ion-exchange properties show potential applications in the treatment of aqueous streams such as the filtration of fruit juices and beverages, where

FIG. 4. Effect of CAN concentration in the pretreatment step on the percent grafting at 25°C. Pretreatment for 2 h in 0.035 N nitric acid solution at 25°C (○). Five hours reaction time in 0.27 N nitric acid (▽).

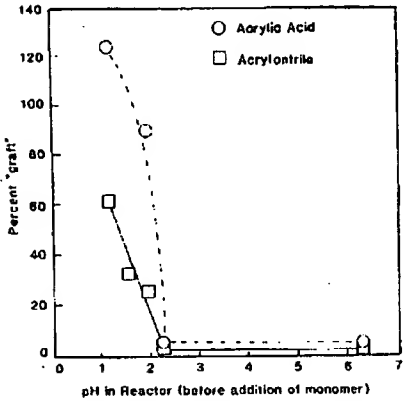

FIG. 5. Effect of pH, before addition of monomer, on the percent grafting at 25°C. Pretreatment as in Fig. 2. Nitric acid concentration varied.

TABLE 2. Grafting of Methacrylic Acid to Paper[a]

CAN concentration, mmol/L	Reaction temperature, °C	Reaction time, h	% "Graft"
20	25	5.0	16
25	25	5.0	14
40	25	5.0	10
20	50	4.0	73
20	50	5.5	99
20	50	7.5	124

[a]Pretreatment conditions: Time = 2 h, temperature = 25°C, nitric acid = 0.035 N. Reaction conditions: Monomer concentration = 5% by volume, nitric acid = 0.27 N.

both ion exchange and filtration can be carried out in one operation. The filter paper has a very low wet tensile strength (15% of the dry tensile strength), and thus improvements in the wet strength are sought for these products for higher operational service life. There are two approaches to solving this problem: 1) wet strength resin-treated paper can be used as a substrate in the graft copolymerization process, and 2) paper can be graft copolymerized with acrylic acid first and then cured with wet strength resin later. The above two approaches have been studied, and the results on the strength improvements will be presented in another paper. The results of the grafting of acrylic acid to a wet strength resin-treated filter paper is described here. Papers were treated with melamine-formaldehyde resin and different levels of weight increase were obtained as described in the experimental section. These papers were graft copolymerized with acrylic acid by the adsorbed ceric ion process, and the results on the effect of melamine resin in the paper on the grafting process are presented in Table 3. It is apparent from Table 3 that the graft yield drops sharply from 150 to 9% "graft" upon treating the filter paper with melamine resin to a content of 0.69%. Richards and White [2] have also observed very poor graft yields in the case of grafting methyl acrylate to urea-formaldehyde resin-treated paper by the conventional ceric ion process. However, it is also apparent from Table 3 that the graft yield increases with an increase in the melamine resin content of the paper.

TABLE 3. Effect of Melamine Resin in Paper on the Graft Copolymerization of Acrylic Acid[a]

Melamine resin in paper, %based on dry paper	Reaction time, h	Monomer concentration, % by volume	% "Graft"
0.00	5	5	150
0.69	5	5	9
3.01	5	5	20
4.38	5	5	78

[a]Pretreatment conditions: CAN concentration = 25 mmol/L, temperature = 25°C, nitric acid = 0.035 N. Reaction conditions: Nitric acid = 0.27 N, temperature = 25°C.

CONCLUSIONS

A viable all-aqueous system for the direct grafting of acrylic acid onto paper and wood pulp has been developed. The process is capable of being exploited industrially. A pretreatment of the cellulose with the ceric salt followed by washing off the excess was used. Methacrylic acid grafts to much lower yields than acrylic acid under the room temperature conditions used.

Paper prepared with melamine resin to confer wet strength properties, essential for ion-exchange use, have also been successfully grafted but with lower yields.

The water sorbing, ion-exchange, and mechanical properties of papers grafted with the technique presented above will be described in Parts II and III of this series.

REFERENCES

[1] A. H. Zahran, J. L. Williams, and V. Stannett, J. Appl. Polym. Sci., 25, 535 (1980).
[2] G. N. Richards and E. F. T. White, J. Polym. Sci., Part C, 4, 1251 (1964).
[3] R. J. E. Cumberbirch and J. R. Holker, J. Soc. Dyers Colour., 82(2), 59 (1966).
[4] O. Y. Mansour and J. Schurz, Sven. Papperstidn., 76, 288, 528, 415 (1973).
[5] A. Gangneux, D. Wattiez, and E. Marechal, Eur. Polym. J., 12, 543 (1970).
[6] D. J. McDowall, B. S. Gupta, and V. Stannett, Am. Chem. Soc. Symp. Ser., 187, 45 (1982).
[7] Y. Ogiwara, H. Kubota, and M. Tanizaki, Paper Presented at 19th High Polymer Conference, Tokyo, 1970.
[8] Y. Ogiwara, Yu. Ogiwara, and H. Kubota, J. Polym. Sci., Part A1, 6, 1489 (1968).
[9] D. J. McDowall, B. S. Gupta, and V. Stannett, Prog. Polym. Sci., 10, 1 (1984).
[10] I. Teraski and M. Matsuki, Sen-i Gakkaishi, 18, 147 (1962).
[11] A. A. Katai, V. K. Kulshrestha, and R. H. Marchessault, J. Polym. Sci., Part C, 2, 403 (1963).

J. MACROMOL. SCI.-CHEM., A22(5-7), pp. 591-618 (1985)

Reactions of Transformation of Polyacrylamide Obtained by Polymerization in Inverse Suspension

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0022-233X/85/2705-0591$3.50/0

...m. Res., 9(5), 165 (1975).
...I., Dokl. Akad. Nauk Az. SSR, 29(10),

..., Ibid., 25(5), 27 (1969).
(1957), Patent Office, London.
(1957), Patent Office, London.
29 (1964).
, and T. Noguchi, *Kobunshi Kagaku*,

...ta, and T. Noguchi *Ibid.*, 25 (274),

. B. Bunger, *Organic Solvents:*
...nd *Methods of Purification Tech-*
Vol. II, Wiley-Interscience, New

...l and *Diene Monomers*, Part I, Wiley-
...rk, 1970.
...ymerization, Part II, Dekker, New

...l and *Diene Monomers*, Part II, Wiley-
...rk, 1971.
...nger, P. Schlack, and F. Sommer-
20(3), 199 (1970).
...ach, and H. Zahn, *Angew. Chem.*,
(1973).
J. Sledz, F. Schue, and G.
Polymer, In Press.
J. Sledz, F. Schue, and G.
Ibid., In Press.

J. MACROMOL. SCI.-REV. MACROMOL. CHEM., C19(2), 193-220 (1980)

Graft Copolymerization of Vinyl Monomers onto Silk Fibers

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I. INTRODUCTION

Modification of the properties of textile fibers in order to get a fiber of improved textile performance is the subject of study of several groups of scientists and technologists [1–4]. Of the several methods available, grafting promises to be a potentially effective means of altering the fiber properties through the added polymer formed *in situ* without destroying the basic properties of the parent fiber. Copolymerization is attractive to chemists as a means of modifying macromolecules since, in general, degradation can be minimized. The desirable properties of the polymer are retained, and copolymerization provides additional properties through the added polymer. The added polymer may be formed *in situ* by polymerization of a monomer or monomers, by condensation of reactants, or by the deposition of preformed polymer.

A variety of property changes can be imparted to silk through grafting without destroying the crystallinity or crystallization potential of the substrate or reducing its melting point. Some of the most dramatic changes in properties which have been brought about by grafting to silk are viscoelasticity, stereoregularity, hygroscopicity, water repellency, improved adhesion to a variety of substances, improved dyeability, settability, soil resistance, bacteriocidal properties, antistatic properties, and thermal stability.

Silk is a protein, a much simpler one resembling α-wool in form [4, 5]. Nearly 60% of the protein chain is made up of the amino acids glycine and alanine to give the repeating unit. X-ray data from Bombyx mori silk and Tussah silk indicate that they consist of extended polypeptide chains, connected in sheets by N—H···O hydrogen bond, with alternate chains oppositely oriented and with the sheets so stacked as to give good close packing of the R groups between the layers.

With the wide variety of vinyl and other available monomers today, grafting promises to be a potentially powerful method for producing substantial modification of fiber properties. Various methods have been used for grafting vinyl monomers onto silk. The most important methods which have attracted attention in recent years are (1) radiation initiation, (2) chemical initiation, and (3) redox initiation.

II. RADIATION INITIATION

Radiation has become an attractive method of producing free radicals suitable for initiating polymerization within a macromolecular substrate [6, 7]. Many new polymer chains can be grafted to the substrate, and it is possible to characterize the copolymer with respect to number, size, and position of the polymer chains. However, although the method is convenient to apply in the laboratory, has general applicability to a variety of substrates, and has received remarkable attention throughout the world, little commercial use has been made of this technique. However, very little has been known about radiation-induced grafting onto silk.

Radiant energy is usually applied to the substrate in the form of high-energy protons (γ-rays) from a radioactive isotope source or as high-energy electrons (β-rays). With irradiation systems such as α-particles, protons, and neutrons, residual radioactivity remains. Cobalt-60 is a convenient source of γ-rays. The predominant effect entering organic substrates as γ-rays is Compton absorption, i.e., an electron is ejected from an atom after collision with another photon of lower energy. The ejected electron may interact with other atoms to raise their energy level to an excited state. If the electron possesses sufficient energy, another electron is ejected, leaving behind a positive ion. The excited atoms and ions can take part in further reactions in the substrate and transfer their energy or decompose into radicals and atoms. In general, it is the free radicals that give rise to polymer formation in the presence of a vinyl monomer.

Arai et al. [8] have reported graft copolymerization onto silk by the high energy radiation technique. Usmanov and co-workers [9] have used the vapor-phase technique for radiation-induced grafting of vinyl fluoride onto natural silk. The physicomechanical properties and morphology of the copolymer were studied. The graft copolymerization was conducted at irradiation doses of 0.5–4.5 Mrad. The rate of grafting has been increased by adding a third material to the system. The graft yield increased to 97.1 and 10.6% in the presence of water and ethanol, respectively, compared to 3.2% in the absence of a polar solvent. The resistance of the silk to corrosive acids was improved on grafting. Grafting also improved the tensile strength and photostability of the silk. A copolymer containing 13.2% vinyl fluoride retained 9.1% H_2O and 9.9% oil, compared to 20.0 and 26.1%, respectively, for the original sample.

The reactions involved in the grafting reaction may be represented as below.

Initiation:

$$S \longrightarrow S^{\cdot}$$

$$S^{\cdot} + M \xrightarrow{k_i} SM^{\cdot}$$

Propagation:

$$SM_n^{\cdot} + M \xrightarrow{k_p} SM_{n+1}^{\cdot}$$

Termination:

$$SM_m^{\cdot} + SM_n^{\cdot} \xrightarrow{k_t} S_{m+n} \text{ or } SM_m + SM_n$$

where S represents the silk polymer chains; $S^{\cdot}$ the silk radicals; M the monomer; $SM_n^{\cdot}$, $SM_{n+1}^{\cdot}$, and $SM_m^{\cdot}$ the graft-copolymer radicals; k_i, k_p, and k_t are rate constants for initiation, propagation, and bimolecular termination.

$$\text{Further, } d[SM_n^{\cdot}]/dt = k_i[S^{\cdot}][M] - 2k_t[SM_n^{\cdot}]^2$$

$$[SM_n^{\cdot}] = (R_i/2k_t)^{1/2}$$

So the expression for R_p will be $R_p = k_p[M][R_i/2k_t]^{1/2}$.

If the chain transfer due to solvent is considered, then the rate expression can be represented in a modified form as follows:

$$S \curvearrowright S^{\cdot}$$

$$S^{\cdot} + F \longrightarrow F^{\cdot} + S$$

$$S^{\cdot} + M \longrightarrow \text{homopolymerization}$$

$$S + F^{\cdot} \longrightarrow S^{\cdot} + F$$

$$F^{\cdot} + M \longrightarrow M^{\cdot} + F$$

$$FM_n^{\cdot} + S \longrightarrow S^{\cdot} + FM_n$$

$$FM_n^{\cdot} + M \longrightarrow M^{\cdot} + FM_n$$

$$M_m^{\cdot} + F \longrightarrow F^{\cdot} + M_m$$

$$FM_0^{\cdot} + S^{\cdot} \longrightarrow \text{termination}$$

$$FM_m^{\cdot} + M_m^{\cdot} \longrightarrow \text{termination}$$

where S is the solvent, M is the monomer, F is the silk, $S^{\cdot}$ is the solvent radical, $M^{\cdot}$ is the monomer radical and $M_m^{\cdot}$ is the homopolymer radical. Hence

$$R_p = k_p[M][R_i]2k_t(R_i + R_{i,h})]^{1/2}$$

where $R_{i,h}$ is the rate of homopolymerization.

III. CHEMICAL INITIATION

In recent years chemical modification of silk through grafting has received considerable interest. This is indeed a very fascinating field of research with unlimited future possibilities for improving the properties of the product. Methods of chemical initiation yield free radicals which are not necessarily part of the substrate, and the covalent bond is only formed between the material and the added polymer by chain transfer. The chemical initiation includes (A) vanadium(V), (B) chromium(VI), (C) cerium(IV), (D) manganese(IV) and manganese(III), (E) peroxydiphosphate, (F) peroxydisulfate, and (G) redox systems.

A. Vanadium(V) Initiation

In the recent past, metal ions in their higher valence states have been extensively used for polymerizing a number of vinyl monomers [10–14]. Quinivalent vanadium was employed [15–17] as initiator of graft polymerization in systems of polymer backbones containing groups such as —CHO, =CO, or $-NH_2$ (capable of being oxidized to free radicals on the backbone) and a suitable monomer (which is grafted). Vanadium has been used for graft copolymerization of methyl methacrylate onto wool [18] and cellulose [19]. This meth-

od of grafting is known to have the advantage that little or no homopolymer is formed since the major problem during the process of grafting is the formation of homopolymer on silk backbone which might be entangled with the silk matrix and be very difficult to remove completely by the usual solvent extraction technique. Neyek and co-workers [20] have reported the graft copolymerization of methyl methacrylate onto silk using quinivalent vanadium ion. The rate of grafting was determined by varying the monomer, the acidity of the medium, the initiator, the temperature, the reaction medium, and the nature of the silk. The graft yield increased significantly by increasing the monomer concentration. The graft yield increased with an increase of $[V^{5+}]$ up to 0.01 M, and beyond this graft the yield decreased. The graft yield was considerably influenced by chemical modification prior to grafting and followed the order as unmodified silk > esterfied silk > trinitrophenylated silk. The amino and carboxyl groups were blocked during the process of acetylation and trinitrophenylation. Free radicals could not be created at the silk backbone by the interaction of the metal ions. As a result, the graft yield decreased.

The probable mechanism may be represented as follows: Vanadium(V) occurs [21] (pH < 1) in the acidity region where it is a useful oxidant as a cation of the simplest formula VO_2^+. The ion may be more correctly written as $[V(OH)_4]^+$ or $[V(OH)_4OH_2]^+$. The redox potential of the vanadium(V)–vanadium(IV) couple increases with acidity in the region from a pH of 1.5 to 2 *N* acid, but rather more steeply than this at higher acidities; presumably the activity of water is reduced at higher acidities and so influences the redox equilibrium:

$$VO_2^+ + 2H^+ + e \rightleftharpoons VO_2^{2+} + H_2O$$

Mishra and Symons [22] excluded the formation of species such as $V(OH)_4^+$ and VO_2^+, but have suggested the formation of such species as $VO(HSO_4)_3$ and $VO(OH)(HSO_4)_2$. Gillespie et al. [23] suggested the formation of $VO(HSO_4)_3$ or $H[VO(HSO_4)_4]$, the latter differing from the former only by a solvent molecule.

The first systematic investigation of the oxidation of organic substrates by quinivalent vanadium was attempted by Waters' school [21].

1. Primary radical production:

$$S + VO_2^+ \overset{K^I}{\underset{\text{fast}}{\rightleftharpoons}} \text{Complex I} \xrightarrow{k_1} R^\cdot + V^{4+}$$

$$S + VO_3^{2+} \overset{K^{II}}{\underset{\text{fast}}{\rightleftharpoons}} \text{Complex II} \xrightarrow{k_2} R^\cdot + V^{4+} + H_3O^+$$

$$S + VO.(OH)^{2+} \overset{K^{III}}{\underset{\text{fast}}{\rightleftharpoons}} \text{Complex III} \xrightarrow{k_3} R^\cdot + V^{4+} + H^+$$

$$S + V(OH)_3HSO_4^+ \overset{K^{IV}}{\underset{\text{fast}}{\rightleftharpoons}} \text{Complex IV} \xrightarrow[\text{slow}]{k_4} R^\cdot + V^{4+} + H_2SO_4 + H_2O$$

where

$$VO_2^+ + H_3O^+ \overset{k_1}{\rightleftharpoons} V(OH)_3^{2+}$$

$$VO_2^+ + H^+ \overset{k_2}{\rightleftharpoons} V(OH)_3HSO_4^+$$

$$VO_2^+ + H^+ \overset{k_3}{\rightleftharpoons} VO.(OH)^{2+}$$

In the presence of a monomer, the free radical $R^\cdot$ starts the chain reaction.

2. Initiation:

$$R^\cdot + M \xrightarrow{k_i} RM^\cdot$$

3. Propagation:

$$RM^\cdot + M \xrightarrow{k_p} RM_2^\cdot$$
$$\vdots$$
$$RM_{n-1}^\cdot + M \xrightarrow{k_p} RM_n^\cdot$$

4. Linear termination by V^{5+}

$$RM_n^{\cdot} + VO_2^{+} \xrightarrow{k_{t_1}} \text{polymer} + V^{4+}$$

$$RM_n^{\cdot} + V(OH)_3^{2+} \xrightarrow{k_{t_2}} \text{polymer} + V^{4+}$$

$$RM_n^{\cdot} + VO(OH)^{2+} \xrightarrow{k_{t_3}} \text{polymer} + V^{4+}$$

5. Reaction of the primary radical with V^{5+}

$$\left.\begin{aligned} R^{\cdot} + VO_2^{+} &\xrightarrow{k_{o_1}} \\ R^{\cdot} + V(OH)_3^{+} &\xrightarrow{k_{o_2}} \\ R^{\cdot} + VO(OH)_3^{2+} &\xrightarrow{k_{o_3}} \end{aligned}\right\} \text{Products} + V^{4+}$$

By applying steady-state conditions, the rate of polymerization can be obtained from the above scheme as follows:

$$R_p = \frac{(k_p/k_t)K[S][M]^2}{[M] + (k_o/k_t)V^{5+}}$$

This scheme is quite reasonable as it agrees with the observed results.

B. Chromium(VI) Initiation

Cr(VI) has been used by Nayak and co-workers [24–26] as a redox initiator for the homopolymerization of vinyl monomers. However, its employment as an initiator for graft copolymerization of vinyl monomers onto textile fiber has not been studied extensively. The feasibility of chromium(VI) to induce graft polymerization of methyl methacrylate onto silk [27] has been reported.

The graft yield increased with increasing monomer concentration up to 0.65 M, and with further increase of monomer concentration the graft yield decreased. The graft yield is proportional to the initiator concentration. The addition of neutral salts such as KCl, NaF, Na_2SO_4, and $MgSO_4$ increased the graft yield. This might be due to the fact that these salts catalyze the propagation step, thereby increasing the grafting efficiency. The grafting reaction is conducted in perchloric acid. The graft yield increases to a certain concentration of acid and thereafter decreases. With increasing acid concentration, the acid anions probably complex with monochromate ion, reducing the oxidizing power of the latter, as pointed out by Stewart and Ice [28] for the variation of oxidation rates of isopropyl alcohol. The percentage graft-on has also been calculated in the presence of the anionic surfactant sodium lauryl sulfate (NaLS) at the critical micelle concentration (CMC). At CMC, micelles are formed which are roughly spherical in size with the sulfate ions forming the Gouy-Chapman double layer [29, 30]. The enhancement of graft yield by NaLS at CMC can be explained on the assumption that the micelles become entangled with the silk fiber as a result of which the chromium(VI) is electrostatically attracted toward the silk matrix. The concentration of Cr^{6+} ion will be greater in the vicinity of the fiber, and free radical formation on the silk backbone will be facilitated, as a result of which the graft yield will increase.

The reactions involved in initiation may be represented as follows. In a system consisting of Cr^{6+}, perchloric acid, MMA, and silk, Cr^{6+} reacts with silk to form silk macroradicals which react with vinyl monomer resulting in the formation of a graft copolymer on the backbone of the fiber:

$$HCrO_4^{-} + S + H^{+} \xrightarrow{k_1} Cr(IV) + \text{product}$$

$$Cr(IV) + S \xrightarrow{k_2} S^{\cdot} + Cr(III)$$

Initiation:

$$S^{\cdot} + M \xrightarrow{k_i} SM^{\cdot}$$

Propagation:

$$SM^{\cdot} + M \xrightarrow{k_p} SM_1^{\cdot}$$
$$\vdots$$
$$SM_{n-1}^{\cdot} + M \xrightarrow{k_p} SM_n^{\cdot}$$

Termination:

$$SM_n^{\cdot} + Cr(VI) \xrightarrow{k_t} \text{polymer} + Cr^{5+}$$

C. Ceric Ion Initiation

The thermal and photochemical reactions involving tetravalent ceric ion in aqueous solution have been the subject of considerable research during the last three decades. Of all the transitional metal ions, ceric salts have been used most extensively for oxidation studies of a multitude of organic as well as inorganic substrates [31–33]. That ceric ions could also initiate polymerization of vinyl monomers was reported by Bacon [34] as early as 1946. Saldick [35] made a qualitative study of the initiating capacities of ceric ion in different acid media. Mino, Kaizerman, and Rasmussen [36] reported the kinetics of vinyl polymerization of acrylamide using the redox system ceric nitrate-3-chloro-1-propanol. Katai et al. [37] investigated the kinetics of polymerization of acrylonitrile initiated by the ceric sulfate-ethylene glycol redox system. Santappa et al. [38–40] and Machida et al. [41–44] have extensively studied the aqueous polymerization of various vinyl monomers initiated by ceric ion redox systems.

On account of its high grafting efficiency compared to other known redox systems, this system has gained considerable importance in grafting vinyl monomers onto cotton and cellulose [46–54] wool [65, 66], collagen [67–69], and nylon 6 [70–72]. Nayak et al. [73] have studied the graft copolymerization of methyl methacrylate onto silk using tetravalent ceric ion. The effect of [monomer], [cerium(IV)], temperature, and the nature of silk on grafting has been discussed. The graft yield increases with increasing monomer concentration up to 0.65 mol/L, and with a further increase of monomer the graft yield decreases. The percentage of grafting increases with increasing ceric ion concentration up to 0.03 mol/L, and thereafter it decreases. The rate of reaction is temperature dependent. The grafting is considerably influenced

by chemical modification of silk prior to grafting. The effect of different species of ceric ion has also been investigated. The graft yields obtained with ceric ammonium nitrate (CAN) are much higher than those obtained with ceric ammonium sulfate (CAS). It has been reported that the rate of dissociation of CAN is much faster than that of CAS [74–76]. Thus the difference in graft yield obtained with both initiators can be ascribed to the greater efficiency of CAN in producing active sites on the substrate backbone compared to CAS. It has been reported that the ceric sulfate complex is more stable than the ceric nitrate complex. Thus the first dissociation of CAN might produce more Ce^{4+} to react with the silk backbone to give rise to more silk macroradicals for which the graft yield increases.

The following reaction scheme has been proposed for the graft copolymerization.

Initiation:

$$SH + Ce^{4+} \xrightleftharpoons{K} \text{complex} \xrightleftharpoons{hv} S^{\cdot} + Ce^{3+} + H^{+}$$

(S = silk and M = monomer)

$$S^{\cdot} + M \xrightarrow{k_i} S{-}M^{\cdot}$$

$$Ce^{4+} + M \xrightarrow{k_i'} M^{\cdot} + Ce^{2+} + H^{+}$$

Propagation:

$$SM_n^{\cdot} + M \xrightarrow{k_p} S{-}M_{n+1}^{\cdot}$$

$$M_m^{\cdot} + M \xrightarrow{k_p'} M_{m+1}^{\cdot}$$

Termination:

$$SM_n^{\cdot} + Ce^{4+} \xrightarrow{k_t} SM_n + Ce^{3+} + H^{+}$$

$$M_m^{\cdot} + Ce^{4+} \xrightarrow{k_t'} M_m + Ce^{3+} + H^{+}$$

Oxidation:

$$S^{\cdot} + Ce^{4+} \xrightarrow{k_o} \text{oxidation products} + H^{+}$$

By applying steady-state conditions to the concentration of $[S^{\cdot}]$ and $[S{-}M^{\cdot}]$ in the above reactions, the overall rate of polymerization can be derived as follows:

$$R_p = \frac{k_p k_i [M]^2}{k_t [Ce^{4+}]} \left[\frac{k_d [S{-}H][Ce^{4+}]}{k_{-d}[Ce^{3+}][H^+] + k_i[M] + k_0[Ce^{4+}]} \right]$$

At low ceric ion concentrations:

$$R_p = k_p k_i [M]^2 \left[\frac{k_d [Ce^{4+}][S{-}H]}{k_{-d}[Ce^{3+}][H^+] + k_i[M]} \right]$$

which shows that with an increase in ceric ion concentrations, the rate of polymerization should increase, which is found to be true. But at higher concentrations of Ce^{4+}, and when $k_t \gg k_p$ and k_i:

$$R_p = \frac{k_p k_i [M]^2}{k_t [Ce^{4+}]} \left[\frac{k_d [Ce^{4+}][S{-}H]}{k_{-d}[Ce^{3+}][H^+] + k_i[M] + k_0[Ce^{4+}]} \right]$$

which shows that the graft yield decreases at higher $[Ce^{4+}]$ concentration.

D. Manganese(IV) and Manganese(III) Initiation

Permanganate ion is known to be a versatile oxidizing agent because of its ability to react with almost all types of functional groups [77]. This ion, coupled with organic substrates, acts as an efficient redox system for the initiation of vinyl polymerization. Palit and co-workers [78–80] and Mishra et al. [81–84] have exploited this field of research using permanganate ion as an initiator. However, its employment as an initiator for graft copolymerization of vinyl monomers onto textile fibers has not been studied extensively.

Hebeish et al. [85] have reported the graft polymerization onto wool using permanganate ion as an initiator. Abdel-Fattah and co-workers have reported [86, 87] the graft copolymerization of methyl methacrylate, acrylonitrile, and acrylic acid onto

nylon 6 using manganese(IV) as the initiator. Recently, Nayak et al. [88] have reported the graft copolymerization of methyl methacrylate onto silk using permanganate as initiator. In the case of MMA, the percentage graft-on increased with increasing permanganate concentration up to 7% but beyond this a decreasing trend was noticed. The maximum graft yield occurred at a permanganate concentration of 15 mEq/L. Grafting has been carried out in the presence of different acids such as perchloric and sulfuric. Regardless of the kind of acid employed, the grafting yield increased with increasing acid concentration and attained a maximum, but fell again at higher concentrations. The effectiveness of the acid followed the order perchloric > sulfuric.

The grafting reaction was also conducted in the presence of methanol and different neutral salts. Methanol concentration was varied from 5 to 50%. The maximum graft yield was obtained at 20% of methanol concentration. The decrease in graft yield may be attributed to the exhaustion of most of the free radicals formed in the oxidation of methanol and/or to termination of the growing polymer chains grafted onto substrate via chain transfer, giving rise to free methanol radicals. The grafting yield was enhanced by increasing the reaction temperature up to 50°C and thereafter decreased. The grafting yield was significantly enhanced with the amount of silk.

A possible explanation for these observations has been advanced in the light of the following mechanism. Uhlig and Teichmann [89] reported that in $KMnO_4$ system in the presence of acid, free radicals are formed through the reduction of manganese(IV) to manganese(III) and/or manganese(II). The creation of free radical may be represented by

$$Mn^{4+} + H_2SO_4 \longrightarrow Mn^{3+} + H^+ + HSO_4^{\cdot}$$

$$Mn^{3+} + H_2O \longrightarrow Mn^{2+} + H^+ + \dot{O}H; \; HSO_4^{\cdot} + H_2O \longrightarrow H_2SO_4 + OH^{\cdot}$$

Free radicals such as $HSO_4^{\cdot}$ and $O^{\cdot}H$ are represented by $R^{\cdot}$ which initiates free radicals:

$$SH + R^{\cdot} \xrightarrow{k_r} S^{\cdot} + RH$$

where SH is silk.

In a system such as manganese(IV), methyl methacrylate, and silk, an intermediate complex of Mn(IV)-silk might be formed and this might dissociate, giving rise to unpaired electrons on the backbone of silk which initiate graft polymerization.

Initiation:

$$SH + Mn^{4+} \xrightarrow{K} \text{complex} \xrightarrow{k_r} S^{\cdot} + Mn^{3+} + H^{+}$$

$$S^{\cdot} + M \xrightarrow{k_i} S\text{—}M^{\cdot};\ Mn^{4+} + M^{\cdot} \xrightarrow{k_i} M^{\cdot} + Mn^{3+} + H^{+}$$

Propagation:

$$SM_n^{\cdot} + M \xrightarrow{k_p} SM_{n+1}^{\cdot};\ M_m^{\cdot} + M \xrightarrow{k_p} M_{m+1}^{\cdot}$$

Termination:

$$SM_n^{\cdot} + Mn^{4+} \xrightarrow{k_t} \text{grafted polymer}$$

$$M_m^{\cdot} + Mn^{4+} \xrightarrow{k_t} \text{homopolymer}$$

Oxidation:

$$S^{\cdot} + Mn^{4+} \xrightarrow{k_o} \text{oxidation product} + Mn^{3+} + H^{+}$$

Applying steady-state conditions to the free radicals, the expression for R_p has been derived as follows:

$$R_p = \frac{k_p k_i k_r [M]^2 [Mn^{4+}][SH^{\cdot}]}{k_t (k_i [M] + k_o [Mn^{4+}])}$$

At low Mn^{4+} concentration,

$$R_p = \frac{k_p k_r [M][Mn^{4+}][SH]}{k_t}$$

These above equations for R_p are in agreement with the experimental results.

E. Peroxydiphosphate Initiation

Among the inorganic compounds containing peroxide bonds, peroxydisulfate, $S_2O_8^{2-}$, is well known. Extensive oxidation studies have been carried out [90, 91]. Studies involving peroxydiphosphate are very few in the literature. The minimum oxidation potential of peroxydiphosphate is −2.07 V while that of peroxydisulfate is −2.01 V. This indicates that the former should be a slightly stronger oxidizing agent than the latter.

Edwards and co-workers [92–94] have investigated the photochemical oxidation of water, ethanol, propan-2-ol, and some metal complexes by peroxydiphosphate. Santappa et al. [95–97] have studied the kinetics of self-decomposition of peroxydiphosphate in aqueous sulfuric acid medium. In a recent paper [96] they reported the kinetics of oxidation of water and pinacol by peroxydiphosphate. The graft copolymerization of methyl methacrylate onto wool [98] using peroxydiphosphate ion as the initiator has already been discussed. Lenka, Nayak, and Mishra [99] reported graft copolymerization onto silk using this ion as initiator. The rate of grafting was determined by varying the monomer concentration, peroxydiphosphate ion, temperature, and solvent. The graft yield increased with increasing peroxydiphosphate ion up to 8×10^{-3} mol/L, and with further increase of peroxydiphosphate ion the graft yield decreased. The graft yield increased with increasing monomer concentration up to 9 wt %. The rate of grafting is temperature dependent. The percentage graft-on increased with an increase of acid concentration up to 0.148 mol/L. The rate of grafting was investigated in the presence of such salts as KCl, NaF, $MnSO_4$, $CuSO_4$, and $LINO_3$. The graft yield increased in the presence of these salts. This might be due to the fact that these salts catalyze the propagation step, thereby increasing the grafting efficiency.

On the basis of experimental observations and in analogy with the mechanism proposed for the photochemical oxidation of water by peroxydiphosphate, the following mechanism may be proposed [97].

In a system consisting of peroxydiphosphate ion, H_2SO_4, MMA, and silk, the following reactions might be taking place:

$$H_4P_2O_8^{2-} + H^{+} \underset{}{\overset{K}{\rightleftharpoons}} H_3P_2O_8^{\cdot}$$

$$H_3P_2O_8^{\cdot} \xrightarrow{\text{slow}} H_2PO_4^{\cdot} + HPO_4^{\cdot -}$$

$$H_2O + H_3P_2O_8^- \xrightarrow{\text{slow}} H_2PO_4^{\cdot} + H_2PO_4^- + O^{\cdot}H$$

$$H_2PO_4^{\cdot} + H_2O \rightleftharpoons H_3PO_4 + O^{\cdot}H$$

$$HPO_4^{\cdot -} + H_2O \rightleftharpoons H_2PO_4^- + O^{\cdot}H$$

The $H_2PO_4^{\cdot}$, $O^{\cdot}H$, and $HPO_4^{\cdot -}$ which are produced during the reaction interact with the groups present in the silk backbone, giving rise to silk macroradicals which react with monomer, resulting in grafting as represented by

Initiation:

$$\sim SH \xrightarrow{k_i} \sim S^{\cdot}$$

$$\sim S^{\cdot} + M \xrightarrow{k_i'} \sim SM^{\cdot}$$

Propagation:

$$\sim SM^{\cdot} + M \xrightarrow{k_p} \sim SM_1^{\cdot}$$

$$\vdots$$

$$\sim SM_{n-1}^{\cdot} + M \xrightarrow{k_p} \sim SM_n^{\cdot}$$

Termination:

$$\sim SM_n^{\cdot} + \sim SM_m^{\cdot} \xrightarrow{k_t} \text{dead polymer}$$

Applying steady-state conditions to the concentrations of [S˙] and [SM˙], the rate of polymerization can be derived as follows:

$$R_p = k_p \frac{k_i^{\frac{1}{2}}}{k_t^{\frac{1}{2}}} [SH]^{\frac{1}{2}}[M]$$

The plot of log R_p vs log [M] is linear and passes through the origin, which supplements the observed fact.

When the concentration of peroxydiphosphate ion is increased, a large number of $H_2PO_4^{\cdot}$, $O^{\cdot}H$, and $HPO_4^{\cdot -}$ radicals are formed.

These radicals interact with the silk radical at several sites which initiate grafting, thereby increasing the graft yield. Beyond 8 $\times 10^{-3}$ mol/L of peroxydiphosphate, the decrease in graft yield may be due to the fact that the abundance of free radicals might terminate the growing chain or the free radicals on the backbone of the silk might be oxidized to give rise to the oxidation product and hence the percentage of graft-on decreases.

The thermal behavior of silk grafted with methyl methacrylate have been studied. The results show that the thermal stability of the samples increased with increasing graft-on percentage.

F. Peroxydisulfate Initiation

Peroxydisulfate ion has been used lately to bring about the oxidation of organic compounds, and the kinetics of some of these processes have been studied by different workers. Work on the oxidation of a variety of organic and inorganic substrates by peroxydisulfate up to 1962 was reviewed by House [90] and Wilmarth and Haim [91]. Recently, the interest in such kinetic studies by peroxydisulfate ion has greatly increased.

The readily available peroxydisulfate ion is an excellent and versatile oxidant for a variety of organic and inorganic compounds. It is one of the strongest oxidizing agents known in aqueous solution. The standard oxidation-reduction potential for the reaction

$$2SO_4^{2-}(aq) \longrightarrow S_2O_8^{2-}(aq) + 2e$$

is estimated to be −2.01 V. Reactions involving this ion, however, are generally slow at ordinary temperatures, and many peroxydisulfate oxidations have been studied kinetically.

That peroxydisulfate ion could initiate polymerization of certain vinyl monomers was reported by Bacon [100] as early as 1946. This is one of the oldest initiating reagents reported in the literature. Morgan [101] reported the polymerization of acrylonitrile initiated by peroxydisulfate with silver nitrate, sodium thiosulfate, and ferous ammonium sulfate as reductant. Kern and co-workers [102] and Whitby et al. [103] reported the kinetics of the polymerization of acrolein initiated by the peroxydisulfate-Ag^+ redox system. Recently, Kagiya [104] and co-workers reported the kinetic features of the redox polymerization of acrolein with potassium persulfate-silver nitrate redox system. Arai and co-workers

[105] and Nayak et al. [106] have studied the graft copolymerization of vinyl monomers onto wool using the peroxydisulfate-lithium bromide redox system and the peroxydisulfate-silver nitrate redox system, respectively. Shiozaki and Nakamura [107] have reported the graft polymerization of 4-acetoxystyrene onto silk in the presence of $(NH_4)_2S_2O_8$. The free radicals like $SO_4^{\cdot-}$ and $OH^{\cdot}$ which are produced initiate grafting. Silk was emulsion grafted with 50% 4-acetoxystyrene in the presence of $(NH_4)_2S_2O_8$ at 70°C for 10–90 min. The percentage grafting increased linearly with time (up to 30 min) after a 5-min induction period and leveled off at 60 min. The grafting efficiency reached 95% in 60 min even when the amount of monomer used was varied from 20–100% of the silk weight. The grafted silk had excellent softness, and its moisture regain decreased rapidly with the percentage grafting increasing to ~38% and almost leveled off after this.

G. Redox Systems

Redox systems [10–14, 24–26] have been extensively used for the homopolymerization of vinyl monomers. In recent years, redox-initiated grafting has attracted the attention of several groups of scientists.

1. Manganese(IV)–Oxalic Acid Initiation

Recently, Panda et al. [108] reported the manganese(IV)-oxalic acid redox initiated grafting of methyl methacrylate onto silk. The maximum grafting was obtained for 5×10^{-3} *M* $KMnO_4$, 1.5×10^{-2} *M* oxalic acid, and 84.59×10^{-2} *M* monomer. The temperature of the grafting reaction was 50°C.

The proposed mechanism for the redox system is

$$SH + Mn(IV) \longrightarrow S^{\cdot} + Mn(III)$$

As suggested by Yost et al. [109], free radicals which are formed initiate grafting:

$$Mn^{4+} + C_2O_4^{2-} \xrightarrow{\text{measurable}} Mn^{3+} + CO_2 + COO^{\cdot-}$$

$$Mn^{4+} + COO^{\cdot-} \xrightarrow{\text{rapid}} Mn^{3+} + CO_2$$

$$Mn^{3+} + 2C_2O_4^{2-} \xrightleftharpoons{\text{rapid}} [Mn(C_2O_4)_2]^{-}$$

$$Mn^{3+} + C_2O_4^{2-} \xrightarrow{\text{measurable}} Mn^{2+} + COO^{\cdot-} + CO_2$$

$$Mn^{3+} + COO^{\cdot-} \xrightarrow{\text{rapid}} Mn^{2+} + CO_2$$

Weiss [110] also suggested the formation of $C_2O_4^{\cdot-}$:

$$Mn^{3+} + C_2O_4^{2-} \longrightarrow C_2O_4^{\cdot-} + Mn^{2+}$$

The free radicals $COO^{\cdot-}$ and $C_2O_4^{\cdot-}$, which are represented by $R^{\cdot}$, initiate grafting:

Initiation:

$$SH + R^{\cdot} \xrightarrow{k_d} S^{\cdot} + RH$$

$$S^{\cdot} + M \xrightarrow{k_i} SM^{\cdot}$$

Propagation:

$$SM^{\cdot} + M \xrightarrow{k_p} SM_1^{\cdot}$$

$$\vdots$$

$$SM_{n-1}^{\cdot} + M \xrightarrow{k_p} SM_n^{\cdot}$$

Termination:

$$SM_n^{\cdot} + Mn^{4+} \xrightarrow{k_t} \text{graft polymer}$$

Oxidation:

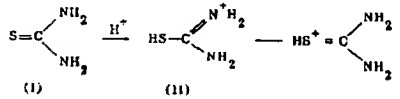
$$S^{\cdot} + Mn^{4+} \xrightarrow{k_o} \text{oxidation products} + Mn^{3+}$$

Applying steady-state conditions to the free radicals, R_p is found to be

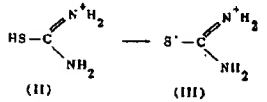
$$R_p = \frac{k_p k_i}{k_t} \frac{k_d[SH][M]^2}{[Mn^{4+}](k_i[M] + k_o[Mn^{4+}])}$$

2. Redox Initiation: Thiourea as Reductant

In all the initiating systems containing thiourea (I), the redox component is isothiourea (II). A thiol (existing in tautomeric equilibrium with thiourea in an aqueous solution) is the reductant. The primary radical is formed by the abstraction of the reactive hydrogen atom attached to the sulfur atom in isothiourea, generating the isothiocarbamido radical (III):

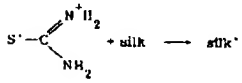


(I) (II)

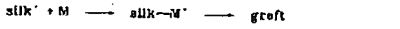


(II) (III)

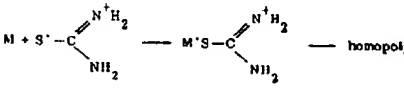
The free radicals attack silk, producing silk macroradicals:



The silk macro radical combines with the monomer, producing grafting:

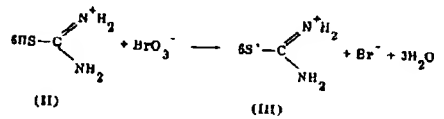


The interaction of the isothiocarbamido radical with the monomer produces the homopolymer:



Potassium bromate is a mild oxidizing agent, and the kinetics of oxidation of organic compounds by potassium bromate has of late, received considerable interest. Hebeish and co-workers [111] have used the potassium bromate-thiourea redox system for grafting methyl methacrylate and methacrylic acid onto nylon 6. Nayak et al. [112] have reported the graft copolymerization of methyl methacrylate onto silk using the potassium bromate-thiourea redox system. The rate of grafting has been determined by varying bromate ion, monomer, and thiourea concentrations, and the temperature and solvent. The graft yield increases with increasing bromate ion concentration up to 20 mmol/L. The percentage grafting increases with an increase of HCl concentration up to 40 mmol/L. The effect of increasing thiourea concentration up to 15 mmol/L was to bring about an increase in the graft yield. The rate of grafting is temperature dependent.

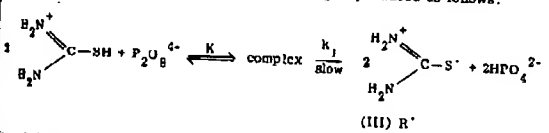
The free radical III is generated as follows:



(II) (III)

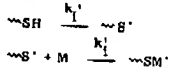
The graft polymerization of methyl methacrylate onto silk using the potassium peroxidiphosphate-thiourea redox system has been reported by Nayak, Lenka, and Mishra [113]. The graft yield increases up to 84.49×10^{-2}, 25×10^{-5}, and 60×10^{-4} mol/L in the case of methyl methacrylate, thiourea, and peroxydiphosphate, respectively. The maximum graft yield was obtained at 50°C.

The free radical which initiates grafting is produced as follows:



(III) R·

Initiation:



Propagation:

$$\sim SM^{\cdot} + M \xrightarrow{k_p} \sim SM_1^{\cdot}$$

$$\vdots$$

$$\sim SM_{n-1}^{\cdot} + M \xrightarrow{k_p} \sim SM_n^{\cdot}$$

Termination:

$$\sim SM_n^{\cdot} + \sim SM_m^{\cdot} \xrightarrow{k_t} \text{dead polymer}$$

Oxidation:

$$\sim S^{\cdot} + \text{oxidant} \xrightarrow{k_o} \text{oxidation product}$$

By applying steady-state conditions to $[R^{\cdot}]$, $[S^{\cdot}]$, and $[SM^{\cdot}]$, the overall rate of polymerization will be

$$R_p = \frac{k_p K^{\frac{1}{2}} k_i^{\frac{1}{2}} [TU][P_2O_8^{4-}]^{\frac{1}{2}}[M]}{k_t^{\frac{1}{2}}}$$

At higher concentration of $[P_2O_8^{4-}]$, the oxidation step may be included and R_p will be

$$R_p = \frac{k_p K^{\frac{1}{2}} k_i^{\frac{1}{2}} k_1^{\frac{1}{2}} [TU][P_2O_8^{4-}]^{\frac{1}{2}}[M]^{3/2}}{k_t^{\frac{1}{2}}[k_i[M] + k_o[P_2O_8^{4-}]]}$$

Pandu et al. [114] have reported the graft copolymerization of methyl methacrylate onto silk using vanadium(V)-thiourea, ferric chloride-thiourea, and hydrogen peroxide-thiourea as redox systems. The initiation step and overall rate of polymerization are as follows:

$$R_p = \frac{k_p k_i^{\frac{1}{2}} K[M]^2[TU]}{k_t^{\frac{1}{2}}[k_i[M] + k_o[V^{5+}]]}$$

$$R_p = \frac{k_p k_i^{\frac{1}{2}} K[M]^2[TU]}{k_t^{\frac{1}{2}}[k_i[M] + k_o[Fe^{3+}]]}$$

$$\xrightarrow{k_1} H_2O + {}^{\cdot}OH + S^{\cdot}-\underset{\underset{NH_2}{\|}}{C}-NH_2$$

$$R_p = \frac{k_p K^{\frac{1}{2}} k_i^{\frac{1}{2}}}{k_t^{\frac{1}{2}}}[TU]^{\frac{1}{2}}[H_2O_2]^{\frac{1}{2}}[M]$$

IV. CONCLUSION

The survival of the natural fibers like silk in competition with synthetics lies in the retention of the fiber as a preferred material in the eyes of the consumer and hence it must be constantly developed and improved to retain that status. If the history of rubber and leather is a guide, textile materials will be gradually displaced by synthetic materials which are amenable to improvement and mass production. This trend has already started.

Therefore, research is needed to maintain the competitive position of the natural fiber over the synthetic fibers. Graft copolymerization is a novel method for improving the properties of silk. This is indeed a fascinating field of research with essentially unlimited future prospects.

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REFERENCES

[1] I. C. Watt, *J. Macromol. Sci.—Rev. Macromol. Chem., C5*, 175 (1970).
[2] P. L. Nayak, *Ibid., C14*, 193 (1976).
[3] P. L. Nayak, *Ibid., C17(2)*, 267 (1979).
[4] K. Arai, *Block and Graft Copolymers*, Vol. 1, Wiley, New York, 1973, p. 193.
[5] H. R. Mauersberger (ed.), *Textile Fibers*, Wiley, New York, 1947.
[6] A. Chapiro, *Radiation Chemistry of Polymeric Systems*, Wiley-Interscience, New York, 1962.
[7] M. Dole, *The Radiation Chemistry of Macromolecules*, Academic, New York, 1973, Vols. 1 and 2.
[8] K. Arai, M. Negishi, S. Komino, and K. Takeda, *Appl. Polym. Symp., 18*, 545 (1971).
[9] Kh. U. Usmanov, A. A. Yulshibaev, A. Valiev, T. Saidaliev, and A. Murutov, *Vysokomol. Soedin., Ser. A. 20(6)*, 1214 (1978).
[10] T. R. Mohanty, B. C. Singh, and P. L. Nayak, *Makromol. Chem. 175*, 2345 (1974).
[11] P. L. Nayak, T. R. Mohanty, and B. C. Singh, *Ibid., 176*, 873 (1975).
[12] R. K. Samal, B. C. Singh, T. R. Mohanty, and P. L Nayak, *Ibid., 176*, 2987 (1975).

[13] T. R. Mohanty, B. C. Singh, and P. L. Nayak, *J. Polym. Sci., Polym. Chem. Ed., 13*, 2075 (1975).
[14] B. C. Singh, T. R. Mohanty, and P. L. Nayak, *Eur. Polym. J., 12*, 371 (1976).
[15] Z. A. Rogovin and R. M. Livshits, *Vysokomol. Soedin, 4*, 784 (1963); Chem. Abstr., 4260 (1964).
[16] R. M. Livshits, R. Mampov, R. G. Zhbankov, and Z. A. Rogovin, *Vysokomol. Soedin. Tsllyulse i Proz. Sb. Statchi.*, p.65 (1963); *Chem. Abstr., 60*, 13429g (1964).
[17] H. Singh, R. T. Thampy, and V. B. Chipalkatti, *J. Polym. Sci., A3*, 1247 (1965).
[18] P. L. Nayak, S. Lenka, and N. C. Pati, *Angew. Makromol. Chem., 71*, 189 (1978).
[19] S. Lenka, P. L. Nayak, and M. K. Mishra, *J. Appl. Polym. Sci. 25*, 1323 (1980).
[20] P. L. Nayak, S. Lenka, and N. C. Pati, *Angew. Makromol. Chem., 68*, 117 (1978).
[21] W. A. Waters and J. S. Littler, in *Oxidation in Organic Chemistry*, (K. B. Wiberg, ed.), 1965.
[22] H. C. Mishra and M. C. R. Symons, *J. Chem. Soc.*, p. 4411 (1962).
[23] R. J. Gillespie, R. Kappoor, and E. A. Robinson, *Can. J. Chem., 44*, 2197 (1966).
[24] R. K. Samal, B. C. Singh, T. R. Mohanty, and P. L. Nayak, *Makromol. Chem., 176*, 2989 (1976).
[25] R. K. Samal, T. R. Mohanty, and P. L. Nayak, *J. Macromol Sci.—Chem., A10*, 1239 (1976).
[26] R. K. Samal and P. L. Nayak, *J. Polym. Sci., Polym. Chem. Ed., 15*, 2603 (1977).
[27] P. L. Nayak, S. Lenka, and N. C. Pati, *J. Appl. Polym. Sci., 23*, 1345 (1979).
[28] R. Stewart and D. G. Lee, *Can. J. Chem., 42*, 439 (1964).
[29] J. H. Fendler and E. J. Fendler, *Catalysis in Micellar and Macromolecular Chemistry*, Academic, New York, 1975.
[30] P. L. Nayak and M. Santappa, *Indian J. Sci. Ind. Res., 35*, 662 (1975).
[31] G. F. Smith, *Cerate Oxidimetry*, Smith Chemical Co., Columbus, Ohio, 1942.
[32] W. A. Waters, *Mechanism of Oxidation of Organic Compounds*, Methuen, 1964.
[33] W. H. Richardson, in *Oxidation in Organic Chemistry* (K. W. Wiberg, ed.), Academic, New York, 1965.
[34] R. G. R. Bacon, *Trans. Faraday Soc., 42*, 140 (1946).
[35] J. Saldick, *J. Polym. Sci., 19*, 73 (1956).
[36] G. Mino, S. Kaizerman, and E. Rasmussen, *Ibid., 31*, 242 (1958).

[37] A. A. Katal, V. K. Kulshrestha, and R. H. Maussault, *J. Polym. Sci., Part C*, *2*, 403 (1963).
[38] S. Venktkrishna and M. Santappa, *Makromol. Chem.*, *27*, 51 (1958).
[39] J. Lalitha and M. Santappa, *Vigyan Parisad Anusandhan Patrica* (Madras), *4*, 139 (1961).
[40] V. S. Ananthanarayan and M. Santappa, *Indian J. Chem.*, *2*, 330 (1964).
[41] H. Narita and S. Machida, *Makromol. Chem.*, *97*, 209 (1966).
[42] H. Narita, S. Okamato, and S. Machida, *Ibid.*, *125*, 15 (1969).
[43] H. Narita, S. Okamato, and S. Machida, *Ibid.*, *14*, 153 (1968).
[44] H. Narita, S. Okamato, and S. Machida, *Ibid.*, *157*, 153 (1972).
[45] G. Landells and C. S. Whewell, *J. Soc. Dyers Colour*, *67*, 338 (1951).
[46] A. Y. Kulkarni and P. C. Mehota, *J. Polym. Sci., Part B.*, *1*, 509 (1963).
[47] S. Kaizerman, G. Mino, and L. F. Meinhold, *Text. Res. J.*, *32*, 138 (1962).
[48] A. Y. Kulkarni, A. G. Chitale, B. K. Vaidya, and P. C. Mehota, *J. Appl. Polym. Sci.*, *7*, 1581 (1963).
[49] E. Schwab, V. Stanetta, D. H. Rakowitz, and J. K. Magrane, *Tappi*, *45*, 390 (1962).
[50] A. Y. Kulkarni and P. C. Mehota, *J. Appl. Polym. Sci.*, *9*, 2633 (1965).
[51] H. Manogawa and T. Sekiya, *Text. Res. J.*, *31*, 585 (1961).
[52] R. H. Cornell, *Tappi*, *45*(7), 145 (1962).
[53] Y. Iwakura, T. Kurosaki, K. Uno, and Y. Imai, *J. Polym. Sci., Part C*, *4*, 673 (1964).
[54] A. Hebeish and P. C. Mehota, *Cellul. Chem. Technol.*, *3*, 469 (1969).
[55] R. J. E. Cumberbirth and J. R. Holker, *J. Soc. Dyers Colour.*, *82*, 59 (1966).
[56] J. C. Arther, B. J. Baugh, and O. Hinojosu, *J. Appl. Polym. Sci.*, *10*, 1951 (1966).
[57] A. Y. Kulkarni and P. C. Mehota, *Ibid.*, *12*, 1321 (1968).
[58] A. Hebeish, A. Kantouch, and M. H. El-Rafie, *Ibid.*, *15*, 11 (1971).
[59] Y. Ogiwara and H. Kubato, *Ibid.*, *17*, 2427 (1973).
[60] Y. Ogiwara, Y. Ogiwara, and H. Kubato, *J. Polym. Sci., Part A-1*, *5*, 2791 (1967).

[61] D. S. Verma and V. Narasimhan, *J. Appl. Polym. Sci.*, *16*, 3325 (1972).
[62] A. Hebeish and P. C. Mehota, *Ibid.*, *12*, 1625 (1968).
[63] A. Hebeish and P. C. Mehota, *Text. Res. J.*, *39*, 89 (1969).
[64] S. Ranga Rao and S. L. Kapur, *J. Appl. Polym. Sci.*, *13*, 2649 (1969).
[66] A. Bendak, A. Kantouch, and A. Hebeish, *Kolor. Ert.*, *13*, 196 (1971).
[67] G. M. Barner and D. J. Termini, *J. Appl. Polym. Sci.*, *17*, 2557 (1973).
[68] K. P. Rao, K. T. Joseph, and Y. Nayudama, *J. Polym. Sci., Part A-1*, *9*, 3199 (1971).
[69] K. P. Rao, K. T. Joseph, and Y. Nayudama, *J. Appl. Polym. Sci.*, *16*, 975 (1972).
[70] D. S. Varma and S. Ravisankar, *Angew. Makromol. Chem.*, *28*, 191 (1973).
[71] D. S. Varma and N. D. Ray, *Ibid.*, *32*, 81 (1973).
[72] D. S. Varma and N. D. Ray, *Ibid.*, *32*, 163 (1973).
[73] P. L. Nayak, S. Lenka, and N. C. Pati, *Ibid.*, *75*, 29 (1979).
[74] T. J. Hardwick and E. Robertson, *Can. J. Chem.*, *29*, 828 (1951).
[75] M. Arden, *J. Chem. Soc.*, p. 1811 (1957).
[76] Z. Reyes, C. E. Rist, and C. R. Russel, *J. Polym. Sci., Part A-1*, *4*, 1031 (1966).
[77] R. Stewart, in *Oxidation in Organic Chemistry* (D. B. Wiberg ed.), Academic, New York, 1984, p. 2.
[78] S. R. Palit and R. S. Konar, *J. Polym. Sci.*, *57*, 809 (1962).
[79] S. R. Palit and R. S. Konar, *Ibid.*, *58*, 85 (1962).
[80] S. R. Palit and R. S. Konar, *J. Polym. Sci., Part A-1*, *3*, 1731 (1964).
[81] G. S. Mishra, J. S. Sukla, and H. Narayan, *Makromol. Chem.*, *118*, 74 (1968).
[82] G. S. Mishra and H. Narayan, *Ibid.*, *113*, 85 (1968).
[83] G. S. Mishra and C. V. Gupta, *Ibid.*, *156*, 195 (1972).
[84] G. S. Mishra and J. J. Rebello, *Ibid.*, *175*, 3117 (1974).
[85] A. Kantouch, S. Abdel-Fattah, and A. Hebeish, *Polym. J. (Jpn.)*, *31*, 375 (1972).
[86] M. I. Khalil, S. H. Abdel-Fattah, and A. Kantouch, *J. Appl. Polym. Sci.*, *19*, 2699 (1975).
[87] S. H. Abdel-Fattah, E. Allam, and M. A. Moharram, *Ibid.*, *20*, 1049 (1976).
[88] P. L. Nayak, S. Lenka, and N. C. Pati, *J. Macromol. Sci.,—Chem.*, *A13*(8), 1157 (1979).

[89] E. Uhlig and N. Teichmann, *Faserforsch. Text. Hoch.*, *20*, 451 (1969).
[90] D. A. House, *Chem. Rev.*, *62*, 185 (1962).
[91] W. R. Wilmarth and A. Haim, in *Peroxide Reaction Mechanism* (J. O. Edwards, ed.), Interscience, New York, 1962, p. 175.
[92] A. A. Green, J. O. Edwards, and P. Jones, *Inorg. Chem.*, *5*, 1858 (1966).
[93] M. Anderson, J. O. Edwards, A. A. Green, and M. D. Wiswell, *Inorg. Chem. Acta*, *3*, 655 (1969).
[94] E. Chaffee, I. I. Creaser, and J. O. Edwards, *Inorg. Nucl. Chem. Lett.*, *7*, 1 (1971).
[95] P. Maruthamuthu, K. V. Seshadri, and M. Santappa, *Indian J. Chem.*, *10*, 762 (1972).
[96] P. Maruthamuthu and M. Santappa, *Ibid.*, *14A*, 35 (1976).
[97] P. Maruthamuthu and M. Santappa, *J. Inorg. Nucl. Chem.*, *37*, 1305 (1975).
[98] P. L. Nayak, S. Lenka, and M. K. Mishra, *J. Appl. Polym. Sci.*, *25*, 63 (1980).
[99] S. Lenka, P. L. Nayak, and M. K. Mishra, *Angew. Makromol. Chem.*, *84*, 183 (1980).
[100] R. G. R. Bacon, *Trans. Faraday Soc.*, *42*, 140 (1946).
[101] M. L. Morgan, *Ibid.*, *42*, 184 (1946).
[102] R. C. Schula, H. Cherdron, and W. Kern, *Makromol. Chem.*, *24*, 141 (1957).
[103] G. S. Whitby, M. D. Gross, J. R. Millen, and A. J. Costanza, *J. Polym. Sci.*, *16*, 544 (1955).
[104] T. Kagiya, S. Morita, and K. Fukui, *Bull. Chem. Soc. Jpn.*, *42*, 2578 (1969).
[105] M. Negishi, K. Arai, S. Okada, and I. Nagakura, *J. Appl. Polym. Sci.*, *9*, 3465 (1965).
[106] P. L. Nayak, S. Lenka, and N. C. Pati, *Angew. Makromol. Chem.*, *85*, 15 (1980).
[107] H. Shiozaki and K. Nakamura, *Nippon Sanshigaku Zasshi*, *46*(5), 451 (1977).
[108] G. Panda, N. C. Pati, and P. L. Nayak, *J. Appl. Polym. Sci.*, *25*, 1479 (1980).
[109] H. F. Lauer and D. M. Yost, *J. Am. Chem. Soc.*, *56*, 2571 (1934).
[110] J. Weiss, *Discuss. Faraday Soc.*, *2*, 188 (1947).
[111] A. Hebeish, M. H. El-Rafie, and A. I. Waly, *J. Polym. Sci., Polym. Chem. Ed.*, *14*, 2895 (1976).
[112] P. L. Nayak, S. Lenka, and N. C. Pati, *Angew. Makromol. Chem.*, *95*, 28 (1980).
[113] P. L. Nayak, S. Lenka, and M. K. Mishra, *J. Polym. Sci.*, In Press.
[114] G. Panda, PhD Thesis, Utkal University, Orissa, India.

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Antibacterial Activity of Polycationic Biocides

TOMIKI IKEDA*

ABSTRACT: Antibacterial activity of polycationic biocides is reviewed in connection with their interaction with their target site, the cytoplasmic membranes of bacteria. The mode of action of low molecular weight cationic disinfectants is first described with reference to the structure of the bacterial cell envelope and then that of the polycationic biocides is discussed on the basis of elementary processes proposed for the low molecular weight analogues. The polycationic biocides discussed include quaternary ammonium salts and biguanides, which are used almost exclusively for disinfection. Application of the polycationic biocides to a variety of fields, such as immobilized biocides and self-sterilizing materials, is finally described.

INTRODUCTION

Polymeric biocides are, in a wide sense, functional polymers and powerful candidates for polymeric drugs, with high activity that can be achieved by their characteristic nature of carrying high local density of the active groups in the vicinity of the polymer chains. The long chain of the polymer can be divided into several parts so as to provide one with some specific group that possesses a high affinity toward a target site. The polymer with this structure can reach the target site easily, thus the local concentration of the drugs at the specific site can be very high. Although synthetic polymers have been used as structural replacements for damaged or diseased human bones and tissues, it is only recently that synthetic polymers with biological activity have received attention. Polymeric drugs are expected to show advantages in terms of localization in specific organs or tissues, reduced toxicity, and increased duration of action [1,2]. However, very few examples with adequate biological activity have so far been discovered [2,3]. This lack of discovery is partly due to bioactive groups often losing their activity when incorporated into a polymer chain.

Polymeric drugs may be divided into two groups. In the first type, bioactive molecules are incorporated covalently into a polymer, thus the polymer chain is used just as a carrier. In the second type, the origin of activity is ascribed to the polymeric form, and these types of polymeric drugs may be termed "intrinsic" polymeric drugs. Polycationic biocides may belong to the first type, in view of the fact that they originate from monomeric or dimeric cationic disinfectants (quaternary ammonium salts and biguanides) with high activity and low toxicity. However, in view of the mode of action, the polymeric form is considered the primary origin of extremely high activity, thereby the polycationic biocides may concurrently be classified into the second type of the polymeric drugs.

In this chapter, we review the antimicrobial activity of low molecular weight cationic disinfectants now widely used all over the world, with special reference to activity–structure relationships. We then give a detailed description of the antimicrobial activity of the polycationic biocides, and discuss the mode of action of the polycationic biocides based on their interaction with the cell envelopes of bacteria, which are considered their target sites.

A characteristic feature of the polycationic biocides is good processability and superior physical properties, in comparison with the low molecular weight analogues. The film-forming property of the polycationic biocides may enable fabrication of "self-sterilizing materials", for which we will find wide use in therapy and hygiene. In the last section of this chapter, we refer to applications of the polycationic biocides.

LOW MOLECULAR WEIGHT CATIONIC DISINFECTANTS

Antimicrobial agents so far in use are classified according to their target sites, as shown in Table 1 [4].

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Table 1. Classification of antibacterial agents according to their target sites [4].

Inhibition of biosynthesis of peptidoglycan in cell walls	β-Lactam antibiotics (penicillins, cephalosporins)
Cytoplasmic membranes disorganization	Phenols (chlorinated cresols etc.) Quaternary ammonium salts (cetrimide etc.) Biguanides (chlorhexidine etc.) Cyclic oligopeptides (tyrocidin A, gramicidin S, polymyxin B etc.)
Change in membrane permeability	Ionophores (valinomycin, nonactin etc.)
Inhibition of biosynthesis of nucleic acids	Azaserine, DON, acridine, actinomycin D, mitomycin, etc.)
Inhibition of biosynthesis of proteins	Puromycin, streptomycin, tetracycline, chloramphenicol etc.

Antibiotics which inhibit biosynthesis of bacterial cell walls, proteins and nucleic acids mainly show bacteriostatic activity, thus preventing the growth of bacterial cells. In contrast, such antimicrobial agents as phenols, quaternary ammonium salts, biguanides and cyclic oligopeptides, whose target sites are the cytoplasmic membranes of microbes, kill microbial cells, exhibiting bactericidal action. However, as has been clinically verified, antimicrobial agents are not necessarily bactericidal in the treatment of microbial infection, since we are provided with antibody and phagocytic defenses which are readily activated to remove bacterial cells from the body. Furthermore, the difference between bacteriostatic and bactericidal is not clearly defined. Many antibacterial agents are known that show bacteriostatic activity at lower concentrations and bactericidal activity at higher concentrations. In Figure 1 are shown the structures of membrane-active antibacterial agents which exert their lethal action by affecting the cytoplasmic membranes. In early remedies, such strong oxidants as chlorine, iodine, and hydrogen peroxide, as well as salts of heavy metals (e.g., mercury) were used, but nowadays their use is limited because of their high reactivity and toxicity.

Currently two main groups of compounds are used almost exclusively for disinfection. They are phenols and cationic disinfectants. Cresols solubilized with soap or alkali are still used, but now their use is rather limited owing to their high toxicity and irritating nature. Hexachlorophene was used widely in surgical soaps. However, its use has been strictly limited after its effect on the nervous system was recognized. Today if we go to hospitals we perceive no smell of phenols. This is because the phenols have been replaced by odorless cationic disinfectants in most of the hospitals. The cyclic oligopeptides (tyrocidin A, gramicidin S, polymyxins, etc.) exhibit high antibacterial activity, but they are of no value from the clinical point of view because of their high toxicity.

Although the structure and the mode of action are different, these membrane-active antibacterial agents are known to show the following common features [4]:

(1) They are easily adsorbed onto bacterial cytoplasmic membranes, and the amount of the adsorbed agents depends on the concentration of the agents. They show similar adsorption isotherms against spheroplasts and protoplasts which are free from the cell walls. Adsorption of these agents onto isolated cell membranes has been confirmed.

(2) Bactericidal action of these agents is dependent on the concentration of the agents, the number of bacterial cells, and the time of contact.

(3) Correlation between their cidal action and leakage of cytoplasmic constituents has been recognized. The low level of the agents induces leakage of low molecular weight cellular constituents like K^+ ions, and higher levels of the agents bring about loss of higher molecular weight solutes such as nucleotides. Loss of the cytoplasmic constituents to some extent is, however, not lethal to the cells. The cells often survive and grow normally when the treated cells are placed in a nutrient medium.

(4) The membrane-active agents are essentially bactericidal, but they show bacteriostatic effect at lower concentrations.

(5) At higher concentrations and upon prolonged exposure, the membrane-active agents penetrate the bacterial cells and cause irreversible damage to the cells.

Use of quaternary ammonium salts (Quats) as disinfectants started early in the 1930s. Domagk found that benzalkonium salts (Figure 1) were outstandingly effective for disinfection of skin and were superior to phenols in killing bacteria [5]. These benzalkonium salts were called "invert soap" or "cationic soap" and have been widely used in disinfection. They still play a role in disinfection of hands and skin and in sterilization of medical equipment. However, their toxicity seems to be somewhat higher than that of the biguanides described below.

Biguanide compounds were first synthesized by Rose et al. of I.C.I. in the mid-1940s. In the early stage, it was mainly the potentiality of biguanides as antimalarial agents that was realized, and some biguanides like proguanil found some practical application in the treatment of malaria [6]. Proguanil is a monobiguanide and is apparently not active in its original structure. The mode of action study has revealed that it becomes an active form (dihydro-

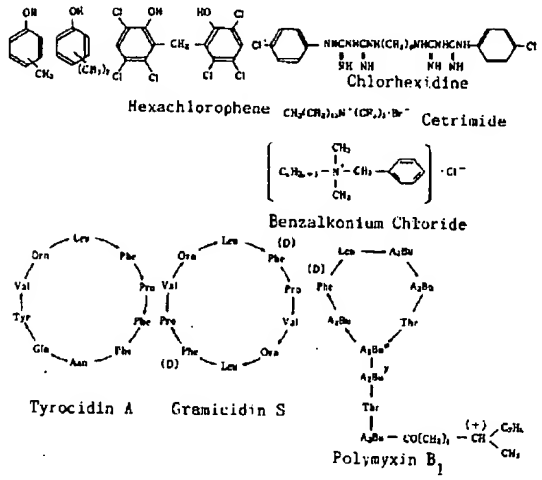


Figure 1. Structures of membrane-active antibacterial agents [4].

triazine) through metabolism in the body. Proguanil was less active against bacteria, but bisbiguanides developed in the mid-1950s by the same group were found to show remarkably high activity [7]. One of the best and most widely used cationic antiseptics is chlorhexidine (Figure 1). The biguanide group involved is one of the strongest organic bases and its pK, value is as high as 12. Thus, at physiological pH it is entirely protonated [8]. Biguanides are generally synthesized in the form of chlorides which are hardly soluble in water. In the preparation of biguanides, the counter-anion is usually changed to a gluconate and the resulting biguanide salts are highly soluble in water (~40%). The biguanide disinfectants have advantages over other disinfectants as follows [4]:

(1) The biguanide disinfectants have a wide spectrum of antibacterial activity against both Gram-positive and Gram-negative bacteria.
(2) The kill rate is extremely high.
(3) Toxicity towards mammalian cells is very low and irritancy is so insignificant that the biguanide antiseptics can be used on the sensitive mucosal surfaces.

Although the cationic disinfectants have a variety of structures, they possess common structural features —positive charge and a fairly hydrophobic part, in a single molecule. For example, in chlorhexidine the positively charged biguanide groups are attached to both ends of a fairly hydrophobic hexamethylene group and in quaternary ammonium salts a hydrophobic alkyl chain is chemically bonded to the positively charged nitrogen atom.

Although a wide range of cationic compounds with these characteristics exhibit more or less antibacterial activity, the activity is strongly dependent on the structure. In the quaternary ammonium salts, the length of the hydrophobic tails has been found to affect the antibacterial activity. For instance, in the analogues of cetrimide (Figure 1) a compound with 14 carbon atoms shows the highest activity, and others with longer or shorter chains exhibit much less. In a series of benzalkonium salts, those of 12 to 14 carbon atoms are known to show the maximum activity. Furthermore, branched hydrocarbon tails reportedly improve the antibacterial activity and are favored for reducing the toxicity [9]. In the analogues of chlorhexidine, the central part of the molecule was found to play an important role. Antibacterial activity was sensitively affected by the length of the alkyl spacers. Thus, in the cationic disinfectants, the hydrophobic parts in the molecule play a significant role, and the hydrophilic–lipophilic balance (HL balance) has been frequently used as a parameter to elucidate their antibacterial activity in some quantitative way.

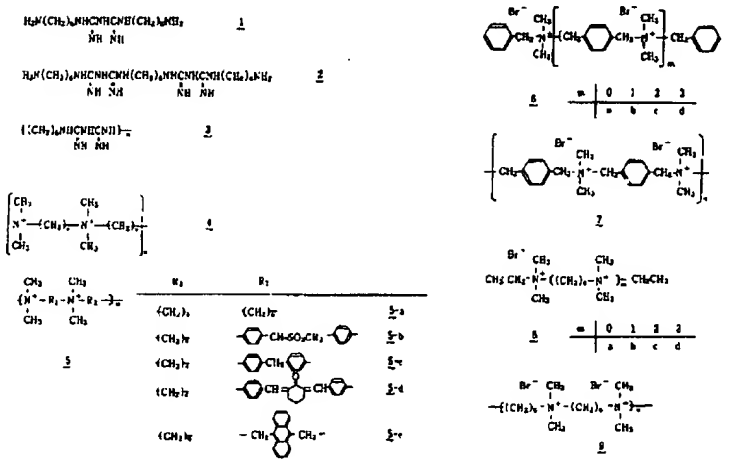


Figure 2. Structures of polycations with main-chain positive charges.

ANTIBACTERIAL ACTIVITY OF POLYCATIONIC BIOCIDES

The most general approach one would take for polymeric drugs may be covalent incorporation of low molecular weight drugs into a polymer. By this procedure, many polymeric drugs have been prepared in which the monomeric drugs were incorporated into a main chain or a side chain. The readers should refer to a review article [10] for detail, but as a conclusion very few successful examples have so far been reported. For example, common antibiotics with β-lactam structure such as penicillins and cephalosporins were polymerized through several routes. However, the resulting polymeric antibiotics were found to exhibit much less activity than the parent antibiotics; in some cases the polymeric forms of the antibiotics lost completely their original activity.

Loss of activity is partly ascribed to the location of the target sites. In case of the intracellular target sites, drugs must overcome an enormous barrier, cytoplasmic membrane, in order to reach their target sites. Because of the molecular size, permeability of the polymeric drugs through the cytoplasmic membrane is reasonably expected to be reduced, thereby making it more and more difficult for the polymeric drugs to reach their target sites inside the cells. Fortunately, however, the target site of the cationic disinfectants is the cell envelope of bacteria, and the reduced permeability, resulting from the increase in molecular size due to polymerization, is not regarded as a factor seriously affecting their activity. In fact, polycationic biocides are one of the rare groups of materials that can effectively utilize the advantages associated with the polymeric form of drugs, such as high local concentrations of active groups at the target site. This section deals with antibacterial activity of polycations having quaternary ammonium salts and biguanides in the main chain or in the side chains.

Polycations with Main-Chain Positive Charges

The structures of polycations with main-chain quaternary ammonium salts or main-chain biguanide groups are shown in Figure 2. These polycations are more or less active against bacteria.

Compound *3* is a polymeric in-chain biguanide, and *1* and *2* are monomeric and dimeric model compounds, respectively. In these compounds, all the biguanide groups are protonated and are hydrochloride salts. The polymeric in-chain biguanide is now commercially available (Vantocil) from I.C.I. and its average degree of polymerization lies in the range 5–7 [11,12]. This polymer shows an outstandingly high antibacterial activity against Gram-positive and Gram-negative strains and possesses a wide spectrum of antimicrobial activity.

Figure 3 shows the log (survivors) versus exposure

time plots for these in-chain biguanide compounds. These plots were evaluated against *Staphylococcus aureus* by the viable cell-counting method. The cells of *S. aureus* at the concentration of $\sim 10^5$ cells/ml were exposed to 10 μg/ml of the biguanide compounds and the number of the surviving cells was counted at various exposure times by the spread plate method. The monomer *1* showed little bactericidal activity. The bactericidal activity of the dimer *2* was higher than that of *1* and 99.9% of the *S. aureus* cells were killed after 2 h exposure. On the other hand, the polymer *3* was so active against this strain that within 10 min all cells were killed. Not only *S. aureus* but also *Escherichia coli*, a typical Gram-negative bacterium, was found to be sensitive to this polycation. Bactericidal activity against *E. coli* also increased in the order of monomer < dimer ≪ polymer [13]. The effect of molecular weight on the bactericidal activity was further examined on the polymeric in-chain biguanide *3* with higher molecular weight than that commercially available. A polymeric biguanide with the degree of polymerization larger than 10 was found to show much higher activity against *S. aureus* and *E. coli* [14].

Polycations with in-chain quaternary ammonium salts also show antibacterial activity [15–20]. Polycations with positively charged nitrogen atoms in the main chains like *4*–*9* are called ionenes. Reinbaum et al. studied the antibacterial activity of ionene *4*, in which the number of methylene spacers, *x* and *y*, was varied [15–17]. Although monomeric and dimeric homologues of *4* could not prevent the growth of bacteria even at such a high concentration as 1,000 μg/ml, the polyionene *4* showed high bacteriostatic activity against *S. aureus* and *E. coli* as shown in Table 2. In the table, the figure indicates the minimum inhibitory concentration (MIC) expressed in μg/ml, which is a measure of the bacteriostatic activity. The growth of bacteria can be seen as colonies in the presence of the drug at the concentrations below the value of MIC. Thus, the lower is the value, the higher becomes the bacteriostatic activity of the drug.

It is clearly seen in the table that the length of the alkyl spacers affects sharply the bacteriostatic activity of the ionenes and 6,10-ionene with $x = 6$ and $y = 10$ exhibited the highest activity against *S. aureus* and *E. coli*. These ionenes reportedly show bacteriostatic activity against *Pseudomonas aeruginosa* and *Bacillus subtilis*. Vucetic et al. explored the antibacterial activity of polyionenes having spacers other than methylene chains (*5*) and reported that *5*-a and *5*-b exhibited bactericidal activity against *S. aureus* and *5*-c–*5*-e were bacteriostatic against the same bacterium [19]. In these early studies, however, no systematic works were performed on, for example, the effect of molecular weight on the antibacterial activity.

Ikeda et al. synthesized a series of ionenes with the same spacer structure but different molecular weights in order to investigate the effect of the molecular

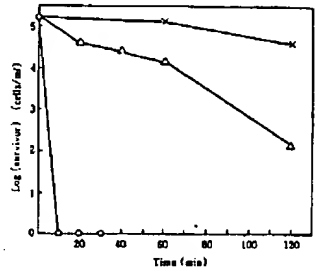


Figure 3. Bactericidal activity of polycation *3* and low molecular weight analogues (*1*,*2*) against *S. aureus* [3] (×), *1*; (△), *2*; (○), *3*. Concentration of the cations was 10 μg/ml.

weight on the antibacterial activity of the cationic biocides [20]. Two kinds of spacer structures were employed. One was a rather rigid xylylene group (*6* and *7*) and the other was a flexible hexamethylene group (*8* and *9*). For both series, low molecular weight homologues [monomer (a), dimer (b), trimer (c) and tetramer (d)] were prepared and were examined as to their antibacterial activity. In Table 3 is shown the bacteriostatic activity of the homologues with xylylene spacers against *B. subtilis*, *S. aureus*, *E. coli*, *Aerobacter aerogenes* and *P. aeruginosa*, as evaluated by the range of the MIC values. The two figures in the table for each strain indicate the range of MIC: the growth of bacteria could be observed as visual colonies below the lower value of MIC, whereas no colonies were seen above the higher value of MIC. Consequently the exact MIC is supposed to lie between the two values. A general trend can be seen in the table indicating that the ionenes are more active against Gram-positive bacteria (*B. subtilis* and *S. aureus*) than against Gram-negative strains (*E. coli*, *A. aerogenes* and *P. aeruginosa*). It is also seen that monomeric (*6*-a) and dimeric (*6*-b) forms are practically inactive against every strain. The striking feature seen in the table is that the activity against *B. sub-*

Table 2. Bacteriostatic activity of various ionenes [17].

Ionene[a]	Minimum Inhibitory Concentration (MIC) *S. aureus* (μg/ml)	*E. coli* (μg/ml)
3,3	125	125
6,6	16	16
6,10	4	4
2,10	4	8
6,16	4	32

[a]The two values indicate the values of *x* and *y* in *4* (Figure 2).

Table 3. Molecular weight dependence of bacteriostatic activity of ionenes [20].

	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Aerobacter aerogenes*	*Pseudomonas aeruginosa*
6-a	>1000	>1000	>1000	>1000	>1000
6-b	>1000	>1000	>1000	>1000	>1000
6-c	600-1000	100-330	>1000	>1000	>1000
6-d	100-330	66-100	660-1000	100-330	>1000
7	33-66	10-33	66-100	66-100	100-330

MIC (μg/m) determined by the spread plate method

tilis and *S. aureus* increases in the order of 6-c < 6-d < 7, i.e., in the order of increasing molecular weight. A similar pattern of the effect of molecular weight on activity can be seen against *E. coli* and *A. aerogenes*. Figure 4 shows the log(survivors) versus exposure time plots for the homologues against *S. aureus* and *E. coli*. The concentration of cations used was 1,000 μg/ml except 7, for which the concentration was 10 μg/ml. The polymer 7 was highly active, and all cells of *S. aureus* and *E. coli* were killed within 30 min of contact. Among the oligomers, the tetramer 6-d was most active against both strains. Although the difference in bactericidal activity among the oligomers with $N^+ < 4$ was not clearly observed against *S. aureus*, the cidal activity was found to increase with molecular weight against *E. coli*. Even at lower concentrations (at 10-100 μg/ml), the tetramer exhibited higher activity than other low molecular weight oligomers.

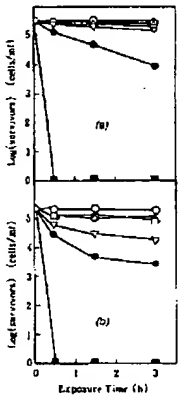


Figure 4. Molecular weight dependence of bactericidal activity of ionenes against *S. aureus* (a) and *E. coli* (b) [20]. (▽), Blank; (△), 6-a; (□), 6-b; (△), 6-c; (●), 6-d; (■), 7. Concentration of the cations was 1,000 μg/ml except 7 of which concentration was 10 μg/ml.

Polycations with Side-Chain Positive Charges

Figure 5 shows the structures of polycations with side-chain positive charges, of which antibacterial activity has so far been recognized. Among these polycations, the potency of antibacterial activity of basic polypeptides was realized in the early stage of studies. In the 1950s, Katchalski et al. found that polyornithine (*10*, $m = 3$), polylysine (*10*, $m = 4$) and polyarginine (*11*) showed antibacterial activity, while their monomeric amino acids were inactive [21,22]. Studies were extended to synthetic basic polypeptides, and polyanthin (*12*), for example, was found to be highly active against *S. aureus*, *B. subtilis* and *Mycobacteria*, although it was practically inactive against Gram-negative species [23,24]. Polyanthin was in fact examined in the clinical application as an antituberculosis agent [23,24].

Panarin et al. synthesized the homopolymers of vinylamine (*18*) and methacrylates with side-chain quaternary ammonium salts (*15*, *16*, *17*) as well as their copolymers with N-vinyl-2-pyrrolidone (*13*, *14*) and examined their antibacterial activity [25]. In *13*-a and *14*-a, various copolymers were prepared in which the compositional ratio of vinylamine or the methacrylate with side-chain quaternary ammonium salt to N-vinyl-2-pyrrolidone was altered, and the effect of the positive charge density on bacteriostatic activity against *S. aureus* was investigated. As shown in Figure 6, a good correlation was observed between the molar fraction of the basic groups in the copolymers and the logarithm of the MIC values of the copolymers. In these copolymers, no effect of the counter-anion on the bacteriostatic activity was recognized among Cl^-, Br^- and I^-. The effect of molecular weight on the bacteriostatic activity against *S. aureus* was also explored for the copolymers *13*-a and *14*-b. In these polycations, the molar fraction of the monomer with the quaternary ammonium salt was kept nearly constant (~25 mol% for *13*-a and ~18 mol% for *14*-b) and the molecular weight was changed as evaluated by the intrinsic viscosity, $[\eta]$, measured in 0.5 M KCl solution. Table 4 shows the MIC values of these copolymers against *S. aureus*,

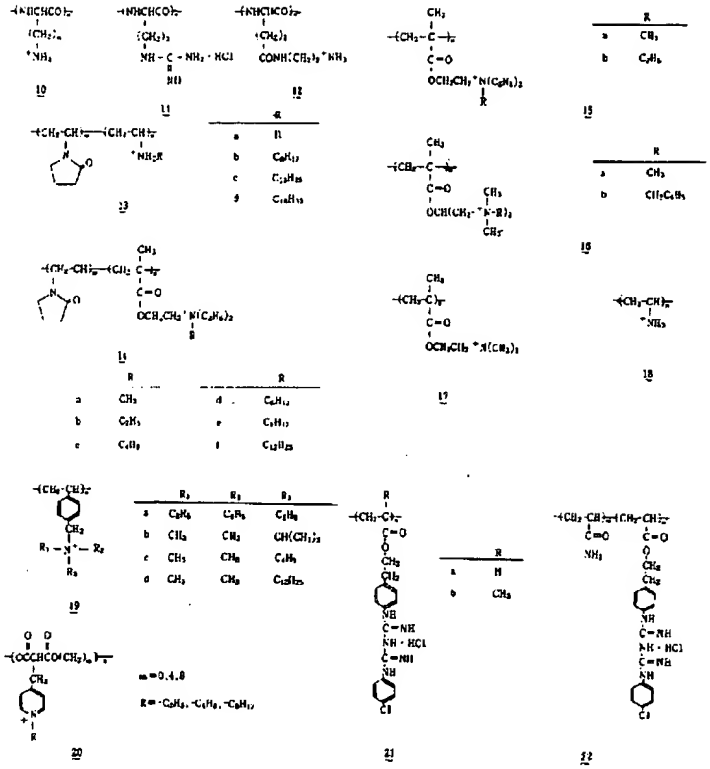


Figure 8. Structures of polycations with side-chain positive charges.

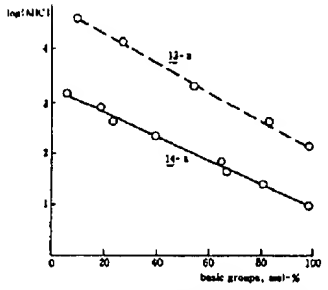


Figure 6. Effect of mole fraction of basic units in copolymers *13*-a and *14*-b on bacteriostatic activity against *S. aureus* [25].

demonstrating that the bacteriostatic activity of the copolymers was not affected by the molecular weight.

Ikeda et al. investigated the antibacterial activity of homopolymers of polyacrylates (*21*-a) and polymethacrylate (*21*-b) with side-chain biguanide groups and their copolymers with acrylamide (*22*) [26,27]. Both of the homopolymers *21*-a and *21*-b exhibited much higher bactericidal activity than those of the relevant monomers. Figure 7 shows the log (survivors) versus exposure time plots for *21*-a (molecular weight ~ 12,000) against *S. aureus*, which was evaluated by the viable cell counting method. Exposure of the *S. aureus* cells (~ 10^8 cells/ml) to the cations was performed in sterile distilled water. In the case of the monomer, ~99% of the bacterial cells were killed after 2 h exposure to 100 μg/ml of the monomeric biguanide, while in the case of the polymeric biguanide (*21*-a), all the bacterial cells were killed within 30 min even at the concentration as low as 0.1 μg/ml [26]. A similar result was obtained against *E. coli*, though the activity against this Gram-negative strain was somewhat lower than against *S. aureus*. The copolymers (*22*) exhibited less antibacterial activity whatever the compositional ratios of the biguanide monomer to acrylamide.

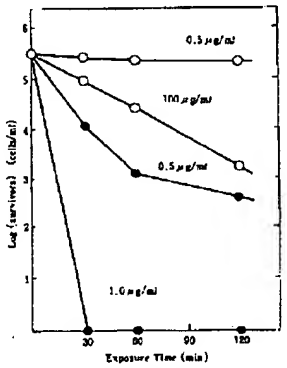


Figure 7. Bactericidal activity of polycation *21*-a and monomer against *S. aureus* [26]. (○), Monomer; (●), *21*-a.

The effect of molecular weight on bactericidal activity was examined on the fractionated samples of *21*-b. Because of the strongly adsorbed nature of the polymeric biguanides towards conventional gel media based on dextran and cross-linked polystyrene, fractionation on the basis of molecular size was only successful by gel filtration with cross-linked acrylamide gel medium. The molecular weight of the fractionated samples was determined with a low-angle light scattering photometer, and those well-characterized samples were examined as to their bactericidal activity against *S. aureus*. As shown in Figure 8, the bactericidal activity of *21*-h was found to be strongly dependent on their molecular weight [27]. A significant result obtained in this study is the presence of the optimal molecular weight region for the cidal action. In the low molecular weight region below molecular weight of 5×10^4, the bacteridical activity increased with molecular weight and in the high molecular weight region above 1.2×10^5 the cidal activity decreased sharply with molecular weight. The polymeric biguanide with the optimal molecular weight exhibited a cidal activity against *S. aureus* more than 10^3 times as high as the monomeric homologue. The molecular weight dependence of the bactericidal activity was also investigated for the polycation with side-chain quaternary ammonium salt (*19*-c). Because of poor polymerizability, the molecular weight of the highest fraction was 77,000 and below this molecular weight the bactericidal activity was found to increase monotonically with molecular

Table 4. Molecular weight dependence of bacteriostatic activity of polycations with side-chain quaternary ammonium salts [25].

Sample	Mole Fraction of Quaternary Ammonium Salts	$[\eta]^a$ (dl/g)	MIC (μg/ml)
13-a	23.5	0.98	300
	24.7	0.89	300
	24.4	0.78	300
	25.2	0.50	300
14-b	18.6	1.12	300
	18.0	0.97	300
	18.8	0.84	300
	18.4	0.73	300

[a] Measured in 0.5 M KCl at 25 °C.

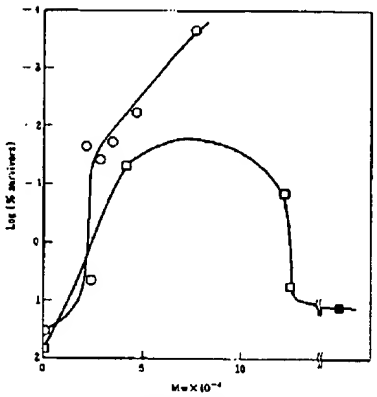


Figure 8. Molecular weight dependence of bactericidal activity of polycations against *S. aureus* [27]. (□), 2*l*-b (10 μg/ml); (○), 19-c (0.5 μg/ml).

weight. Bacteriostatic activity of the fractionated polymeric quaternary ammonium salt was explored against *S. aureus*, *B. subtilis*, *E. coli*, *A. aerogenes* and *P. aeruginosa* and the MIC values obtained showed little molecular weight dependence.

Special precaution must be taken in the evaluation of the antibacterial activity of the polycationic biocides. As Katchalski already pointed out in the early 1950s, polycations, in particular those with high molecular weights, have a strong tendency to form insoluble complexes with polyanions such as DNA and RNA [22]. This strong interaction of the polycations with negatively charged species is considered to be an origin of the high antibacterial activity of the polycationic biocides as discussed in detail in a later section, since the interaction of the polycations with negatively charged components present in bacterial cytoplasmic membranes is regarded as a crucial step in their lethal action. However, the strong tendency to form insoluble complexes with the polyanionic species makes it very difficult to evaluate precisely the antibacterial activity of the polycationic biocides.

Conventionally, bacteriostatic activity of disinfectants is evaluated by the spread plate method. In this method, a bacterial culture is spread on nutrient agar containing various concentrations of the disinfectant, then incubated. It is examined on the formation of visual colonies. Application of this conventional method to polycations, however, requires special precautions. Growth media used to cultivate bacteria usually contain acidic constituents, such as sodium caseinate, which form insoluble complexes with polycations, thus leading to inactivation of the polycationic biocides [27,28]. In fact, this inactivation of the polycationic biocides has been observed so far for the side-chain polycations (*19* and *2l*), and their MIC values against *B. subtilis*, *S. aureus*, *E. coli*, *A. aerogenes* and *P. aeruginosa*, evaluated by the spread plate method, were higher than those of the relevant monomers. In order to eliminate the interference by the constituents in the growth media, the antibacterial assessment should be performed in media free from acidic components. One way to fulfill this requirement is to conduct the exposure of bacterial cells to the polycations in sterile water with subsequent viable cell counting.

The HL balance described in the low molecular weight cationic disinfectants also affects the antibacterial activity of polycationic biocides. In *14*, the substituent at the quaternary ammonium salt (R) was changed from CH_3 (a) to $C_{12}H_{25}$ (f), and the bacteriostatic activity of those copolymers was examined [25]. In Table 5, bacteriostatic activity of the monomers and the copolymers (*m/n* = 75/25) of *14* is shown by the MIC values against *S. aureus*. It is seen that a monomer with a less hydrophobic group (e.g., CH_3) is practically inactive (MIC > 10,000 μg/ml). However, with increasing hydrophobicity of the substituent, i.e., with increasing chain length of the alkyl substituent, the MIC value decreases in a series of the monomers. On the other hand, the MIC values of the copolymers was not significantly affected by the substituents. Furthermore, the copolymers a–d exhibited higher activity than the relevant monomers with the same alkyl group.

Similar behaviors have been observed for polycat-

Table 5. Effect of alkyl chain length in copolymer 14 on bacteriostatic activity against S. aureus [25].

Sample	R	$[\eta]^a$ (dl/g)	MIC (μg/ml) Polymer	MIC (μg/ml) Monomer
14	CH_3	1.05	50	10,000
m/n = 75/25	C_4H_9	1.05	50	1,000
	C_6H_{13}	0.00	50	1,000
	C_8H_{17}	0.80	50	1,000
	$C_{10}H_{21}$	0.72	50	100
	$C_{12}H_{25}$	0.54	45	1

[a] Measured in 0.5 *M* KCl at 25°C.

ions with side-chain quaternary ammonium salts (*19*). In *19*, alkyl chain length was varied and their bacteriostatic activity as well as that of the monomers were explored by the spread plate method [28]. The MIC values thus determined against *S. aureus*, *B. subtilis*, *E. coli*, *A. aerogenes* and *P. aeruginosa* are listed in Table 6. Among dimethylbenzyl ammonium salts (*19*-b, c, d), bacteriostatic activity of the monomers increased in the order of b < c < d irrespective of strain. The activity of the polymers was found to be in the same order against Gram-positive strains. However, the polymer with the longest alkyl chain (*19*-d) exhibited much less activity against Gram-negative bacteria than the other polymers. This polycation (*19*-d), however, showed the highest antibacterial activity when the activity was explored in sterile distilled water—the cidal activity of the polymer was much higher than that of the corresponding monomer [28]. A similar effect of the HL balance on the antibacterial activity was observed for polymeric pyridinium salts (*20*) [29].

Factors affecting the antibacterial activity, other than the molecular weight and the HL balance, include the structure of the spacers between the positive charges. In the polyionene *4*, the methylene spacers between the positively charged N atoms were found to affect the antibacterial activity of the polyionenes in such a way that the longer the methylene spacer is, the higher becomes the activity [20]. Furthermore, a rigid spacer seems to be favored for the antibacterial activity. The polyionene with rigid p-xylylene spacers (*7*) exhibited much higher antibacterial activity than one with flexible hexamethylene spacers (*9*) [20]. However, a polyionene with o-xylylene spacers showed rather lower activity [20]. Hydrophilic spacers were found to reduce the activity. A polyionene with a hydroxy group in the spacer was practically inactive, demonstrating that the hydrophilic spacers drastically reduce the antibacterial activity [20].

In this section, we described the antibacterial activity of various polycations. We have realized a general trend indicating that the polycations are more active than the relevant monomeric compounds, and the polycations show higher activity against Gram-positive bacteria than against Gram-negative strains. We also have pointed out that special precaution should be paid to evaluate the antibacterial activity of polycations with high molecular weight in media containing acidic components. Complexation, followed by inactivation of the polycations, always takes place, making the precise evaluation of the antibacterial activity very difficult.

STRUCTURE OF BACTERIAL CELL WALL AND CYTOPLASMIC MEMBRANES

Before we discuss the mode of action of the polycationic biocides, we refer to the structure of cell envelope of bacteria, which is considered as the target site of the polycationic biocides.

Bacteria are unicellular microorganisms composed essentially of cell wall, cytoplasmic membrane and cytoplasm. Bacteria are classified into two groups depending on the structure of their cell walls; Gram-positive and Gram-negative bacteria. The cell wall of Gram-positive strain is mainly composed of peptidoglycan and teichoic acid (Figure 9) [30]. The peptidoglycan is an alternating copolymer of N-acetylglucosamine and N-acetylmuramic acid, to which polypeptide with appropriate chain length is attached, thus the overall structure of the cell wall of Gram-positive bacteria is somewhat mesh-like [31]. Teichoic acid is a phosphate diester of glycerol or ribitol, therefore is negatively charged at physiological pH, and is considered to play a role in uptake of Ca^{2+} and Mg^{2+} ions [30].

The structure of the cell wall of Gram-negative bacteria is much more complicated than that of Gram-positive species. As shown in Figure 10, the pepti-

Table 6. Bacteriostatic activity of polycation 19 [28].

	S. aureus	*B. subtilis*	*E. coli*	*A. aerogenes*	*P. aeruginosa*
19-a	66–100	100–330	660–1000	660–1000	>1000
Monomer	100–330	330–660	>1000	>1000	>1000
19-b	66–100	100–330	660–1000	660–1000	>1000
Monomer	>1000	330–660	>1000	>1000	>1000
19-c	33–66	66–100	330–660	660–1000	>1000
Monomer	660–1000	660–1000	>1000	>1000	>1000
19-d	33–66	10–33	>1000	>1000	>1000
Monomer	<1	<1	10–33	10–33	66–1000

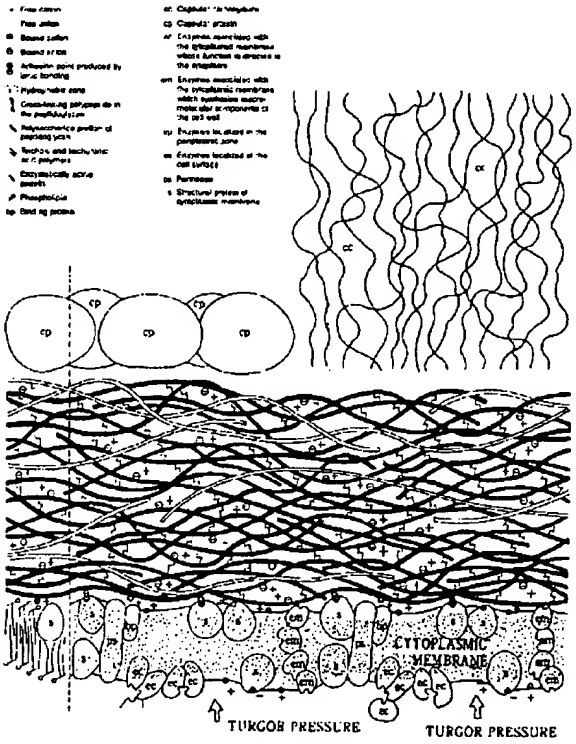


Figure 9. Structure of cell envelope of Gram-positive bacteria [31].

doglycan layer is rather thin, but there is another layer outside the peptidoglycan layer called the outer membrane. The outer membrane is composed mainly of lipopolysaccharides and phospholipids [31]. A significant role of the outer membrane is to protect a bacterial cell from attack by foreign compounds such as disinfectants. Thus, the much lower sensitivity of Gram-negative bacteria toward antibacterial agents is due mainly to the presence of the outer membrane. As described in the previous section, Gram-negative strains are less sensitive toward cationic disinfectants than Gram-positive strains. Furthermore, it is believed that the outer membrane prevents penicillins and lysozyme from reaching their target sites, which is why these antibacterial agents are inactive against Gram-negative bacteria. Removal of metal ions by chelating agents like EDTA results in partial breakdown of the outer membrane since the metal ions are essential to stabilize the lipopolysaccharide layer in the outer membrane. In the presence of such chelating agents as EDTA, penicillins and lysozyme become highly active against Gram-negative species [32].

Unlike the cell wall, the structure of the cytoplasmic membranes is essentially the same between Gram-positive and Gram-negative bacteria (Figures 9 and 10). The main constituents of the cytoplasmic

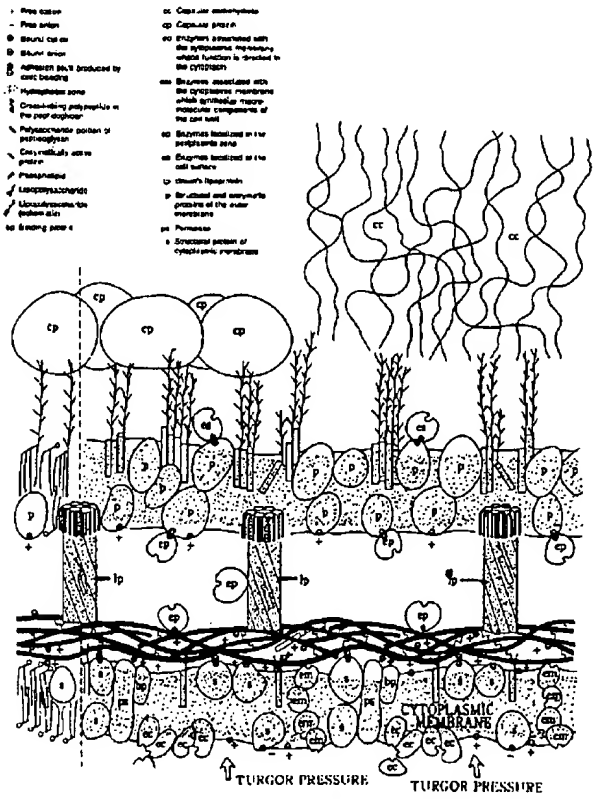


Figure 30. Structure of cell envelope of Gram-negative bacteria [30].

membrane are proteins (membrane proteins) and phospholipids. Extensive studies on phospholipids in the cytoplasmic membrane have been performed, and it has become evident that phosphatidylethanolamine (PE), almost neutral at physiological pH, is a major component present in the bacterial cytoplasmic membrane, and that phosphatidylglycerol (PG) and its dimer cardiolipin (DPG), both of which are negatively charged at physiological pH, are major acidic components. It appears that in the cytoplasmic membrane of *E. coli* PE constitutes about 80% of the total lipids, and the acidic PG and its dimer DPG are each present to the extent of ~ 10% (Table 7) [33,34]. In eukaryotic cells, the phospholipids present in the cytoplasmic membranes are different from those of the prokaryotic cells. In the eukaryotic cells, phosphatidylcholine (PC), neutral at physiological pH, is the major zwitterionic lipid and phosphatidylserine (PS) is the major acidic component in place of PG and DPG of the prokaryotic cells.

Table 7. Composition of phospholipids in cytoplasmic membrane and outer membrane of E. coli [33].

Phospholipid	Cytoplasmic Membrane	Outer Membrane
PE	63.4%	78.0%
PG	10.6	11.0
DPG	6.3 9.3	6.3
Lyso PE	9.4	< 1.0
Unknown	7.3–10.3	4.7

MODE OF ACTION OF POLYCATIONIC BIOCIDES

The target site of the low molecular weight cationic biocides is the cytoplasmic membranes of bacteria and the following elementary processes have been proposed for their mode of action [4,11].

(1) Adsorption onto the bacterial cell surface
(2) Diffusion through the cell wall
(3) Binding to the cytoplasmic membrane
(4) Disruption of the cytoplasmic membrane
(5) Release of cytoplasmic constituents such as K^+ ions, phosphate ions, DNA and RNA
(6) Death of the cell

It is now generally accepted that the mode of action of the polycationic biocides can be interpreted on the basis of each elementary process described above, since the same physiological events as in processes (1), (3) and (5) have been observed for the polycationic biocides.

It is well known that the bacterial cell surfaces are usually negatively charged, as evidenced by the electrophoretic mobility measurements. This is explained by the fact that there are many negatively charged species in the cell wall, as is briefly described in the previous section. The adsorption of polycations onto the negatively charged cell surfaces is expected to be facilitated in comparison with that of monomeric cations because of much higher charge density carried by the polycations. In fact, immediate adsorption of the side-chain polycations (*13–18*) onto the bacterial cell surfaces has been confirmed by fluorescence spectroscopy [25]. Polycations are superior to monomeric cations in the amount and the degree of adsorption [35].

Low molecular weight cationic biocides induce leakage of K^+ ions, phosphate ions and cytoplasmic constituents which have absorbance at 260 nm (mainly DNA and RNA, and called thereafter "260-nm absorbing materials") from the bacterial cells immediately after the cationic biocides are adsorbed onto the cell surfaces [11,36–38]. Figure 11 shows the amounts of K^+ ions, phosphate ions and the 260-nm absorbing materials released from $\sim 10^9$ cells/ml of *E. coli* in contact with 0.2 mM of cetrimide (Figure 1) at 25°C at pH 7 as a function of the contact time [38]. Release of K^+ ions is very fast. It starts soon after the *E. coli* cells are exposed to the cationic biocide and is completed within ~60 min. Loss of the phosphate ions and the 260-nm absorbing materials from the cells is rather slow. Since it has been confirmed that the low molecular weight cationic biocides undergo no interaction with isolated cell wall components, it is reasonably assumed that the low molecular weight cationic biocides penetrate the cell wall and reach the cytoplasmic membrane very quickly, inducing the leakage of the cytoplasmic constituents [39].

A similar leakage of the cytoplasmic constituents from the bacterial cells was observed when the polycations were exposed to bacterial cell culture [14,20,25,27]. Broxton et al. investigated the leakage of the cytoplasmic constituents from the *E. coli* cells ($\sim 10^9$ cells/ml) in contact with various concentrations of the in-chain biguanide polymer (*3*; $n > 10$) and obtained the results shown in Figure 12 [14]. A

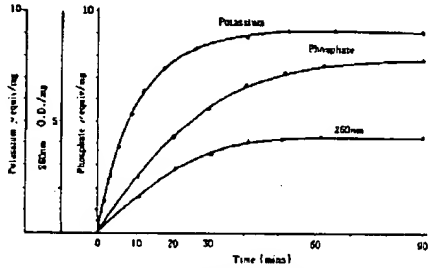


Figure 11. Release of cytoplasmic constituents from *E. coli* cells in contact with cetrimide [38]. [Cetrimide], 0.2 mM; *E. coli*, 10^9 cells/ml; pH, 7; 25°C.

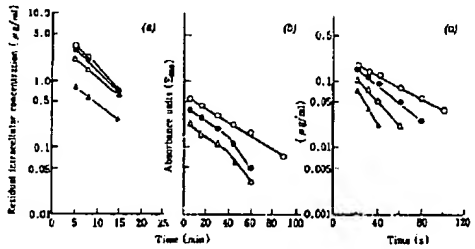


Figure 12. Release of cytoplasmic constituents from *E. coli* cells in contact with polycation *3* [14]. (a) Phosphate ions; (b) 260 nm-absorbing materials; (c) K^+ ions. Concentration of *3* (μg/ml): (▽), 4; (●), 3; (△), 2; (▲), 1 (b): (○), 5; (●), 3; (△), 1.5 (c): (□), 0.4; (●), 0.3; (▲), 0.1 Concentration of the *E. coli* cells was 10^9 cells/ml.

characteristic feature seen in Figure 12 is that the loss of K^+ ions from the cells is extremely fast and is caused by the polymeric biguanide at concentrations much lower than that required for leakage of the phosphate ions and the 260-nm absorbing materials. These results strongly suggest that polycations penetrate the cell wall and reach the cytoplasmic membrane, inducing the leakage of the cytoplasmic constituents as in the case of the low molecular weight cationic biocides.

Morphological change of bacterial cells on exposure to cationic biocides was investigated by electron microscopy [11,25,39]. Davies et al. revealed that when the *E. coli* cells were exposed to a low concentration of a biguanide biocide, chlorhexidine (Figure 1), bacterial cell surface became swollen and blistered, whereas exposure of the bacterial cells to a high level of chlorhexidine brought about leakage of the cytoplasmic constituents, followed by intracellular precipitation of the lost materials [11]. Exposure to the high level of chlorhexidine led finally to shrinkage of the bacterial cells with electron dense bodies inside the cells [11]. A similar morphological change of the *S. aureus* cells was observed when the cells were in contact with a polycation with side-chain quaternary ammonium salts (*15*-b) [25]. These results indicate that the target site of the cationic biocides, regardless of the molecular weight, is the cytoplasmic membrane of bacteria. Strong interaction with the cytoplasmic membrane is a primary step in the morphological change of the cells observed.

In the lethal action of the cationic biocides described previously, process (2) is undoubtedly suppressed as the molecular size of the diffusing species increases, since the peptidoglycan layer in the cell wall acts as a potential barrier against foreign molecules with high molecular weight. As schematically illustrated in Figures 9 and 10, the rigid peptidoglycan layer constitutes the basic framework of the cell walls and provides the bacterial cells with characteristic shapes like a rod and a sphere. The cell wall plays a key role in preventing the bacterial cells from osmotic lysis [40]. Since the rigid peptidoglycan layer possesses a mesh-like structure, foreign molecules with small size are expected to diffuse rather freely through the cell wall. However, diffusion through the cell wall is believed to become difficult for molecules with increasing molecular size [31]. Very few quantitative data are available at present on the size of the foreign molecules that can diffuse through the cell wall without difficulty. It seems reasonable to assume that the size of the freely-diffusing foreign molecules is strain-dependent.

The bell-shaped dependence of antibacterial activity on molecular weight of the polycationic biocide (Figure 8) can be interpreted on the basis of the elementary processes in the lethal action of the cationic biocides [27]. Because of increasing charge density of the polycation, the adsorption of the polycation onto the bacterial cell surfaces is enhanced with increasing molecular weight of the polycation. A similar enhancement can be expected in the binding of the polycation to the cytoplasmic membrane (process 3), since there are many negatively charged species present in the cytoplasmic membrane, such as acidic phospholipids and some membrane proteins (see the section on "Structure of Bacterial Cell Wall and Cytoplasmic Membranes"). The disruption of the membrane (process 4) is a consequence of interaction of the bound polymers with the membrane and is expected to be facilitated with increasing amounts of the bound polymers. Process 4 would be immediately followed by processes 5 and 6, and thus processes 1, 3 and 4 (5 and 6) can be assumed to be enhanced with increasing molecular weight of the polymers. On the other hand, as discussed above, process 2 is supposed to be suppressed with increasing molecular weight. The observed optimal molecular weight region for antibacterial activity against *S. aureus* can, thus, be interpreted in terms of a sum of two kinds of controlling

factors: one is positive (enhanced) with molecular weight (processes 1, 3 and 4) and the other is negative (suppressed) with molecular weight (process 2).

In order to explore the effect of the cell wall, antibacterial activity of the polycationic biocides against two types of bacterial cells was investigated [27]. One is the intact cell and the other is a protoplast which is freed from the cell wall. A protoplast is a bacterial cell which can be prepared by the action of lysozyme in hypertonic solution. Because of the absence of the cell wall, the cytoplasmic membrane is directly exposed to the environment in the protoplast, thus it is quite vulnerable to the change in the environment. The protoplast survives only in the hypertonic solution. The protoplast and the intact cells of *B. subtilis* were exposed to the polycations (*19*-c) with various molecular weights in the hypertonic solutions and the loss of the 260-nm absorbing materials from the both cells was followed [27]. The loss of the 260-nm absorbing materials is in fact a direct measure of cell lysis. Figure 13 shows the amount of 260-nm absorbing materials that were released from the intact cells and the protoplasts of *B. subtilis* in contact with 10 μg/ml of the polycation (*19*-c), as a function of the molecular weight of the polycation exposed [27]. A bell-shaped release profile was obtained for the intact cells, while a monotonic increase was observed for the protoplasts. These results clearly indicate that the target site of the polycationic biocides is the cytoplasmic membrane of bacteria, and exclusion at the cell walls operates for polymers with high molecular weight.

It is still ambiguous how the polycationic biocides interact with the cytoplasmic membrane with subsequent disruption. There are two possible sites in the cytoplasmic membrane for interaction with the polycations: the membrane-bound proteins and the phospholipids. Relatively little is known about the membrane-proteins. On the other hand, the phospholipids have been extensively studied. This may be due partly to the fact that method for isolation and purification of the phospholipids has been nearly established and highly purified samples are available. Furthermore, such excellent model systems as liposome, black lipid membrane and monolayer membrane can now be readily prepared, and their properties have been thoroughly investigated. By the use of these models for the cytoplasmic membranes, many studies on the interaction of various substrates with lipid bilayer membranes have been performed so far.

As described in the section on "Structure of Bacterial Cell Wall and Cytoplasmic Membranes", many kinds of the phospholipids are seen in the bacterial cytoplasmic membranes. Simply, they are classified into two groups according to the structure of the polar head groups. One is zwitterionic phospholipids, which include PC and PE. They are nearly neutral at physiological pH. The other is acidic phospholipids (PG, DPG and PS, etc.) which are negatively charged at physiological pH. With the aid of highly purified, well-characterized phospholipids, the effect of inorganic ions such as Ca^{2+} and Mg^{2+}, organic ions such as acetylcholine and polymyxin B on the model bilayer membranes has been investigated by thermal analysis (DSC etc.), fluorescence spectroscopy, NMR, X-ray diffraction method, and a variety of phenomena have been observed—phase separation, fusion, phase transition to hexagonal II phase and interdigitated phase [41–45].

Tirrel et al. studied the interaction of the polymeric in-chain quaternary ammonium salts with lipid bilayer membranes by DSC and X-ray diffraction. No significant effect of polyethyleneimine on a neutral bilayer composed of dipalmitoylphosphatidylcholine (DPPC) was observed, while on a negatively charged bilayer membrane composed of dipalmitoylphosphati-

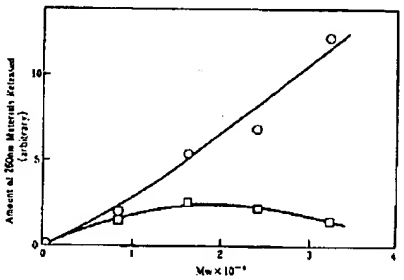


Figure 13. Release profile of 260 nm-absorbing materials from intact cells (□) and protoplasts (○) of *B. subtilis* in contact with polycation *19*-c as a function of molecular weight [27]. Concentration of *19*-c, 10 μg/ml.

diglycerol (DPPG) strong interaction was recognized and the gel-to-liquid crystalline phase transition temperature (T_c) of the negatively charged bilayer was shifted to lower temperatures [46,47].

A specific effect of polyionene (*4*) was observed on the DPPG bilayer membrane. X-ray diffraction study on the structure of the mixture of the DPPG membrane and the polyionene *4* revealed that the interdigitated phase was induced by the addition of the polyionene where the hydrocarbon tails of DPPG are deeply interpenetrated [48,49].

The interaction of the biguanide polymer (*3*) and lipid bilayer membranes was investigated by DSC and fluorescence polarization method [50,51]. The polymer *3* and the monomer model compound *1* both lowered the T_c of the negatively charged bilayer composed of acidic PG by -10°C. This is in contrast to the effect of Mg^{2+} and Ca^{2+}, which increased the T_c of the same bilayer membrane. Behavior of the polymer different from that of the monomer was observed toward a mixed bilayer of PC and PG. The polycation caused aggregation of the negatively charged PG in the vicinity of the adsorption site and formed the polycation/PG domain, thus inducing the phase separation in the mixed bilayer. On the other hand, no aggregation was observed on addition of the monomeric cation. As described in the section on "Structure of Bacterial Cell Wall and Cytoplasmic Membranes", bacterial cytoplasmic membranes are composed of neutral and negatively charged phospholipids, therefore it is believed that polycationic biocides induce phase separation in the bacterial membrane on binding.

Aggregation behavior of acidic phospholipids in the bilayer membranes by basic polypeptides has long been studied. These studies were devoted primarily to elucidating how the basic membrane-proteins interact with matrix phospholipids in the cytoplasmic membranes and how the functions of the membrane-proteins are affected by the matrix phospholipids. These studies are, however, useful in providing the basic insight into the polycation/membrane interaction.

Polylysine-induced phase separation was observed in the mixed bilayer of PC and phosphatidic acid (PA), as in the case of polycation *3*. This result led to the assumption that extrinsic membrane-proteins affect the composition of the phospholipids near the location site, although the extrinsic proteins are only loosely bound at the surface of the cytoplasmic membranes [52]. Spin-labeling study revealed that cytochrome C, an extrinsic membrane protein, specifically binds to the acidic DPG in the mixed membrane of DPG and steroid, and forms a cluster in the membrane [53]. Myelin, a protein containing 38 basic residues in the molecule, induced aggregation of 27–34 molecules of the acidic phospholipids in the vicinity of the location site, and caused phase separation in the mixed bilayer membranes of neutral PC and acidic lipids (PA, PG or PS) [54,55].

Galla et al. investigated the interaction of polymyxin B, a cationic oligopeptide having high antibacterial activity (Figure 1), with the bilayer membrane by the fluorescence polarization method. They found that polymyxin B binds specifically to acidic phospholipids (PA and PG) and induces phase separation in the dipalmitoylphosphatidic acid (DPPA)/distearoyl PC mixed membrane [44]. On addition of polymyxin B to the monolayer of DPPA, expansion of the monolayer was observed. In the case of the bilayer membrane of DPPA, T_c was found to be lowered by -20°C on addition of polymyxin B. However, no effect was observed when polymyxin B was added to the neutral bilayer membrane.

Recently, interaction of a polymeric in-chain ammonium salt with phospholipid bilayer membranes was studied by time-resolved fluorescence spectroscopy in connection with the antibacterial activity of the polycation [56]. Particular attention was paid in this study to a phenomenon of polycation-induced fluidization of the membranes, which was well-evaluated by the time-resolved fluorescence anisotropy measurements. The fluorescence anisotropy, $r(t)$, of 1,6-diphenyl-1,3,5-hexatriene (DPH) embedded in the membranes was analyzed based on the simplest wobbling-in-cone model. Strong interaction was observed between the polycation *7* and the DPPA membrane as demonstrated by a large decrease of residual polarization value on adding the polycation *7* to the DPPA membrane. This means that the cone angle of the wobbling-in-cone motion of the DPH molecule increases by the addition of the polycation *7*, indicating the polycation-induced fluidization of the acidic membrane. On the other hand, $r(t)$ of DPH embedded in the DPPC membrane was not affected significantly by the addition of *7*, which is presumably due to non-binding of the polycation to the zwitterionic membrane. These results clearly indicate that the polycationic biocide interacts strongly with negatively charged membranes, inducing fluidization of the membranes.

Interaction of various polyionenes with phospholipid bilayer membranes was also explored by means of DSC with special reference to their antimicrobial activities [57]. Addition of polyionene *7* and *9* caused phase separation in the mixed bilayer of PC and PA. Ability to induce phase separation was found to depend strongly on the structure of the polyionene. Polyionene with rigid spacer (*7*) was most effective in inducing phase separation and was most active in antimicrobial activity, while polyionenes with rigid and flexible spacers in the alternate fashion exhibited lower activity and their mode of interaction with bilayers was similar to those of all flexible spacers. This result suggests that the rigid spacers are favorable for strong interaction with the negatively charged bilayer membranes, leading to the higher activity. Other factors affecting the mode of interaction with membranes were molecular weight and hydrophobicity. With increasing molecular weight, both activity and ability to induce phase separation in-

creased. Introduction of hydrophilic groups into the spacers resulted in a loss of activity and ability to induce phase separation. The antimicrobial activity and the mode of interaction with membranes were correlated and were found to be interpreted on the basis of the conformational concept of the polyionenes in solution.

Polycationic biocides bind to the bacterial cytoplasmic membranes and disorganize the membrane structure. This will undoubtedly affect the function of the membrane-bound enzymes. It is reasonably expected that the change in activity of the membrane-bound enzymes would be lethal and would result in the death of the bacterial cells. However, very few studies have been performed so far on the effect of the polycations on the membrane-bound enzymes. Panarin et al. investigated the effect of polycations *15* and *16* on the activity of bacterial enzymes activating the antibiotics chloramphenicol and canamycin, which are known to be bound to the cytoplasmic membrane [25]. Chloramphenicolacetyltransferase (CAT) conducts acetylation of hydroxy groups of the antibiotic and transforms it into an inactive diacetyl derivative, and canamycintransferase (CT) also transforms canamycin into an inactive form. It was found that the polycations *15* and *16* significantly inhibit the membrane-bound enzymes (CAT and CT) and the activity of the enzymes was reduced by 10% – 100% by the action of the polycations. The effect of inhibition was dependent on the strain and the structure of the polycations. *S. aureus* was much more sensitive than *E. coli* and polycation *16*-b was most effective.

Replacement of counter-cations of a polyanion is another function of the polycation. This leads to polyanion/polycation complexation and insolubilization of the complex. Replacement of Mg^{2+} and Ca^{2+} ions present at the surface of the bacterial membranes by the polycations is reasonably expected and may result in change in the function of the membrane-bound enzymes. For example, Mg^{2+} and Ca^{2+} are required for ATPase to exhibit its function, thus removal of these inorganic cations would lead to loss of the function of this enzyme [58,59]. Anyhow, the polycation/membrane-bound enzyme interaction is one of the most important subjects to be solved in the future for deeper understanding of the mode of action of the polycationic biocides.

POLYMERIC MATERIALS WITH ANTIBACTERIAL ACTIVITY

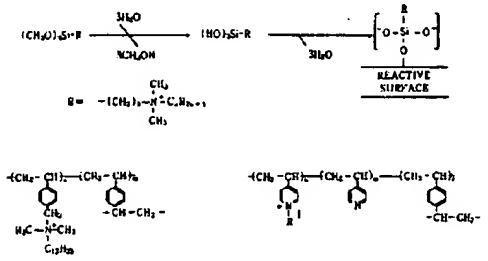
Polymeric drugs are provided with properties superior to those of low molecular weight analogues in view of the high local density of active groups and the film-forming property, which originate from polymeric forms. Furthermore, cross-linking will give the polymeric drugs insoluble features, thus immobilization of the active groups can be readily achieved. Polycationic biocides possess high positive charge density and excellent processability, and have found remarkable utility in hygiene and in biomedical applications.

Biomedical contamination of polymeric materials with microorganisms is a primitive but still quite serious problem and all polymeric materials are subjected to sterilization by means of steam, chemicals and radiation. However, most materials are often re-exposed to the atmosphere, which can lead to recontamination with microorganisms. Antibiotics are prescribed to the patients to prevent microbial infections, and this treatment has in general been successful. However, this treatment suffers from the disadvantage that frequent and excessive use of antibiotics produces resistant bacterial cells, which then require more "powerful" antibiotics to be effective. One possible way to overcome this problem is to develop polymeric materials which themselves have antimicrobial activity. Such materials with intrinsic antimicrobial activity may be called "self-sterilizing materials" (SSM). In this section, application of the polycationic biocides to a variety of fields is described.

Immobilized Polycationic Biocides

Many trials have been conducted so far to provide surface antibacterial activity with polymeric materials by incorporating antibacterial agents covalently onto the surfaces of the polymeric materials. This type of antimicrobial agent is termed an "immobilized" biocide and exhibits its activity through contact with microbial cells, thus this action is sometimes called "contact disinfection". Merits of the "immobilized" biocides are evident. Firstly, because of immobilization of the active groups, contamination of environment with the biocides can be prevented. Secondly, continuous treatment of bacterial cell suspension is possible by the use of column packed with the immobilized biocides. Thirdly, owing to covalent bonding to the matrix media, the immobilized biocides can be regenerated by washing with appropriate solvent, so that they can be used for a long time.

Isquith et al. treated various surfaces with 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride (Si-QAC), a coupling agent, and examined the antimicrobial activity of the surfaces of the materials, which retain the chemically bonded Si-QAC [60]. The ammonium salts were not released from the surfaces by repeated washing with water and showed antimicrobial activity against a wide range of microorganisms. For example, covalent coupling of Si-QAC to glass beads resulted in active particles and only 1–4 cells of *Streptococcus faecalis* were seen on the surface of the treated glass beads while $\sim 10^5$ cells could be observed on the surface of the untreated (blank) glass beads. The surface activity was found to persist after repeated washing with detergent solution, and 50-times washings did not affect the activity. Materials other than glass (natural fibers, man-made fibers and metals) were treated by the same procedure



Siliceous surfaces	Man-made fibers	Metals
Glass	Acrylic	Aluminum
Glass wool	Modacrylic	Stainless steel
Sand	Polyester	Galvanized metal
Stone	Cellulose acetate	
Ceramic	Rayon	Miscellaneous
	Acetate	Leather
Natural fibers	Amides	Wood
Cotton	Spandex	Rubber
Wool	Vinyl	Plastic
Linen	Dacron	Formica
Pelt	Viscose	

Figure 14. Structure of immobilized polycationic biocides and Si-QAC-treated substrates exhibiting antimicrobial activity [60,62,64,66,68].

and the treated surfaces exhibited a similar surface activity [60,61].

Nakagawa et al. prepared similar surface-treated glass beads and explored their surface activity. They used 3-chloropropyltrimethoxysilane as a coupling agent, and quaternary ammonium salts were introduced covalently at the surfaces of the glass beads by the reaction of the chloropropyl groups with various N,N-dimethylalkylamines [62]. They found that the alkyl chain length strongly affected the surface activity. The cell suspension of *E. coli* (~ 10^8 cells/ml) was eluted through a column packed with 1.2 g of the surface-treated glass beads (80–120 mesh) and the cell concentration of the eluates was evaluated. The glass beads retaining C_3-C_6 alkyl chains showed lower activity while those with C_7-C_{18} alkyl chains exhibited high activity. In particular, the glass beads with C_{16} alkyl chains showed the highest activity and contact of the bacterial cell suspension with these glass beads only for 10 s was enough to remove all the cells from the eluates.

Currently, the formation of trihalomethanes and other carcinogens as a result of water disinfection with chlorine is a serious problem. Removal of bacterial cells from water by the use of the immobilized biocides can thus be an alternative and sophisticated method of water disinfection.

Recently, many products are commercially available, which are imparted with surface antimicrobial activity. These include underwear, socks and sheets. Most of the products are those treated with alkoxysilane coupling agents having quaternary ammonium salts at the surfaces, thereby their surface activity is said to remain unchanged after repeated washing [63].

The surface activity of these treated materials is mainly due to adsorption of bacterial cells. The bacterial cells captured seem to be alive on the surfaces, thus the surface activity may not be bactericidal but bacteriostatic. As described in the section on "Mode of Action of Polycationic Biocides", the positive charges play a key role in the adsorption of the cells, but other factors seem to affect strongly the adsorption behaviors.

Cross-linked quaternary ammonium salts have also been studied as to their ability to adsorb the bacterial cells. The cross-linked resins prepared from cross-linked poly(chloromethylstyrene) and N,N-dimethyldodecylamine showed antibacterial activity against *B. subtilis* and exerted an ability to capture 9×10^8 cells/g of the cells [64]. Furthermore, the resin was found to exhibit the activity against viruses [65].

Systematic studies on the effect of the alkyl chain length and the degree of cross-linking on the ability of capturing the bacterial cells were performed for cross-linked poly(pyridinium salts). Nakagawa et al. investigated the adsorption behaviors of the resins pre-

pared from cross-linked poly(4-vinylpyridine) and alkyl iodide [66,67]. In the preparation of the resins, the mole fraction of the cross-linking agent, divinylbenzene, was changed from 10% to 70%. Among the resulting resins, the ability to capture the bacterial cells increased in the order of 70% < 10% < 30% < 50%. For the resins with similar degrees of cross-linking, those with the alkyl chain length of C_9–C_{12} exhibited the highest activity. Kawabata et al. reported that benzyl moiety was quite effective for the capture of various bacterial cells when incorporated into the cross-linked poly(vinylpyridinium salts) [68]. Figure 15 shows the number of the viable cells of *E. coli* in the aqueous phase as a function of the contact time between the cross-linked poly(vinylpyridinium salts) and the benzyl moiety. In the absence of this resin, no change in the number of viable cells was observed, whereas in the presence of this resin the number of the viable cells decreased with time and 99% and 99.99% of the *E. coli* cells were captured after 2 h and 6 h contact, respectively. The capture ability of this resin against the *E. coli* cells was estimated to be $1.2–1.5 \times 10^{10}$ cells/g. This resin was found also to be effective against bacteriophages [69].

The capture ability of the bacterial cells was strongly strain-dependent. Kawabata et al. investigated the cell-capture ability of cross-linked poly(N-benzyl-4-vinylpyridinium salts) and found that the sensitivity to the cross-linked resin increased in the order of *Salmonella* < *Klebsiella* < *Bacillus* < *Enterobacter* < *P. aeruginosa* < *E. coli* < *S. aureus* [70]. The surface negative charges of these bacterial cells were determined to be in the range of 9×10^{-15} 6×10^{-14} g·eq/cell and were dependent on the strains. However, no correlation was recognized between the sensitivity and the surface charges of the cells. These results suggest that factors other than the surface charges of the cells may operate in the adsorption of the cells by the cross-linked polymeric quaternary ammonium salts. Hydrophobicity of the cell surfaces is again strain-dependent. It was revealed by the Rosenberg method [71,72] that in *Staphylococcus*, *Salmonella*, and *Bacillus* the cell surfaces are highly hydrophobic, and among these strains good correlation was observed between the amount of adsorbed cells and the surface charges [70].

Other factors affecting the adsorption behaviors are the cell concentration, flow rate, particle size of the resins and temperature. It was found that low concentration of the cells and low flow rate are favorable for cell capture [66]. Furthermore, small particle size (large surface area) and high temperature seem to be effective [64,66].

Cross-linked polymeric quaternary ammonium salts described so far are inactivated by adsorption of the microbial cells and polyanions. However, the surface activity can be regenerated by removal of the adsorbed materials by washing the resins with ethanol and sodium hydroxide solution. Thus, these cross-linked materials can be reused repeatedly [62,64,

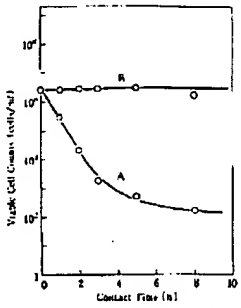


Figure 15. Capture of *E. coli* cells by cross-linked polymeric quaternary ammonium salts [68]. (a) Resins with quaternary ammonium salts, (b) resins without quaternary ammonium salts.

66,68]. Another application of these resins is based on the fact that the adsorbed microbial cells are alive on the surfaces, although the cell division is inhibited. Thus, the cross-linked polymeric quaternary ammonium salts can carry a large number of microbial cells alive on the surfaces and these immobilized microbial cells can be used in bioreactors [70]. This method of immobilization of the microbial cells has advantages over other methods in that it involves simple operations, no chemical reagents which may damage the cells, and has a high strength of adsorption. In fact, alcoholic fermentation by the use of the adsorbed yeast cells on the surfaces of the cross-linked polymeric quaternary ammonium salts has been tested extensively [70].

Polymeric Materials Releasing Low Molecular Weight Biocides

The characteristic feature of this type of SSM is bactericidal activity of their surfaces since biocides are released at their surfaces.

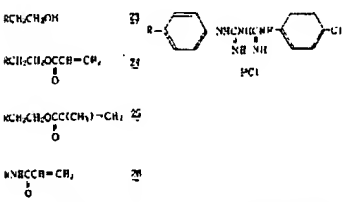


Figure 16. Structures of vinyl monomers with biguanide units in the side-chain (24–26) and released biocide (hydrolyzed product, 23) [76].

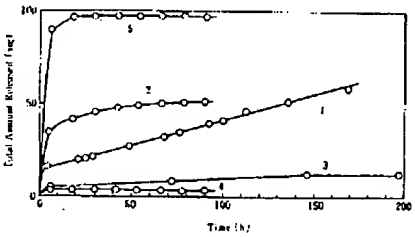


Figure 17. Release of biguanide biocide from cross-linked polyacrylamide films [76]. 1, Containing covalently-bonded *24*; 2, containing homopolymer of *24* physically; 3, containing covalently-bonded *25*; 4, containing covalently-bonded *26*; 5, containing *23* physically.

When the low molecular weight quaternary ammonium salts were retained in the cation-exchange resins as counter ions, they were liberated slowly and exhibited bactericidal activity [73–75]. Liberation of the quaternary ammonium salts was found to be enhanced when the cation-exchange resins prepared from weak acids were employed. Furthermore, dimethylphenylbenzyl ammonium chlorides were easily released regardless of the kind of resins. On the other hand, in the cation-exchange resins prepared from strong acids the amount of released cationic biocides was small. Extensive studies were performed on the combination of AIRC-50 (cation-exchange resin) and cetylpyridinium chloride (cationic biocide). This resin with the biocide was found to be highly effective in killing microbial cells, and no surviving cells were observed in the eluates. However, the bactericidal activity of this resin was again dependent on the cell concentration, contact time and temperature. At higher temperatures the activity was high, but even at room temperature the activity was high enough to kill all microbial cells under ordinary conditions. pH did not affect the activity between 5–14 while culture media such as beef extract and polypeptides were found to reduce the bactericidal activity. The amount of the released biocide was found to be in the range of 1.2–1.6 μg/ml.

The cross-linked polyacrylamide films containing covalently-bonded biguanide biocide (*24*) exhibited bactericidal activity at their surfaces and successfully acted as SSM [76,77]. This cross-linked film was found to be hydrolyzed at the acrylate group in contact with water to release the biguanide biocide (*23*), and when the degree of cross-linking was high, the release was approximately zero-order at least over a period of one week. The rate of release then decreased gradually, but the release was observed to continue for up to a month. Furthermore, the dose of the biocide released could be controlled by the amount of *24* in the preparation stage of the cross-linked film. When the cross-linked films were placed on nutrient agar plates, inoculated with cell suspensions of *S. aureus* and *E. coli* and incubated, no colonies of the bacteria were seen on the plates, whereas bacterial growth was observed as thick colonies on the blank films. Furthermore, the growth inhibitory zone was observed for the cross-linked films, which indicates that the diffusion of the free biocide (*23*) occurs over a relatively long distance [77]. Scanning electron microscopy studies revealed that the inoculated cells of *E. coli* and *S. aureus* underwent morphological changes such as shrinkage and deformation, which is ascribed to the collapse of the bacterial cells due to the bactericidal action of *23*. This SSM film could be regenerated by washing with alcohol and it showed the same surface activity. This type of SSM would be particularly valid in such applications as wound covers and artificial skin for temporary uses.

Another polymeric film with high physical strength was reportedly bactericidal. This is a blend of polyethylene and a copolymer of ethylene and acrylic acid which contains benzalkonium biocide as counter cations [78]. This polymer film was shown to be bactericidal at the surfaces due to the release of the benzalkonium salts in contact with water.

This chapter is dedicated to the late professor Shigeo Tazuke who gave the author outstanding suggestions, but unfortunately died on July 11, 1989.

REFERENCES

1. Donaruma, L. G. and O. Vogl, eds. 1978. *Polymeric Drugs*. New York: Academic Press.
2. Vogl, O. and D. Tirrell. 1979. *J. Macromol. Sci. Chem.*, A13:415.
3. Verlander, M. S., J. C. Venter, M. Goodman, N. O. Kaplan and B. Saks. 1976. *Proc. Natl. Acad. Sci. USA*, 73:1009.
4. Franklin, T. J. and G. A. Snow. 1981. *Biochemistry of Antimicrobial Action*. London: Chapman and Hall, Chapter 3.

5. Domagk, G. 1935. *Deut. Med. Wochenschr.*, 61:829.
6. Curd, F. H. S. and F. L. Rose. 1946. *J. Chem. Soc.*, p. 729.
7. Rose, F. L. and G. Swain. 1956. *J. Chem. Soc.*, p. 4422.
8. Kurzer, F. and E. D. Pitchfork. 1968. *Fortsch. Chem. Forsch.*, 10:375.
9. Hopp, J. O. and A. M. Lands. 1947. *J. Pharmacol. Exper. Therap.*, 79:321.
10. Batz, H.-G. 1977. *Adv. Polym. Sci.*, 23:26.
11. Davies, A., M. Bently and B. S. Field. 1968. *J. Appl. Bacteriol.*, 31:448.
12. Boardman, G. 1969. *Food Technol. N.Z.*, 4:421.
13. Pemberton, D., P. M. Woodcock and T. Ikeda. (unpublished results).
14. Broxton, P., P. M. Woodcock and P. Gilbert. 1983. *J. Appl. Bacteriol.*, 54:345.
15. Rembaum, A., H. Rile and R. Somoano. 1970. *J. Polym. Sci.*, B8:457.
16. Rembaum, A. 1973. *Appl. Polym. Symp.*, 22:299.
17. Rajaraman, R., D. E. Rounds, S. P. S. Yen and A. Rembaum. 1975. "Effects of Ionenes on Normal and Transformed Cells", in *Polyelectrolytes and Their Applications*. A. Rembaum and E. Selegny, eds. Reidel.
18. Ottenbrite, R. M. and G. R. Myers. 1973. *J. Polym. Sci. Polym. Chem. Ed.*, 11:1443.
19. Vucetic, J. J., V. H. Vandjel and M. D. Janic. 1977. *Glas. Hem. Drus. Beograd.*, 42:389.
20. Ikeda, T., H. Yamaguchi and S. Tazuke. 1990. *J. Bioact. Comp. Polym.*, 5:31.
21. Katchalski, E., L. Bichovski Slomnitzki and B. E. Volcani. 1953. *Nature*, 169:1095.
22. Katchalski, E., L. Bichovski-Slomnitzki and B. E. Volcani. 1953. *Biochem J.*, 55:671.
23. Kovacs, K., A. Kotai and I. Szabo. 1960. *Nature*, 185:266.
24. Kovacs, K., A. Kotai, I. Szabo and R. Meeseki. 1961. *Nature*, 192:190
25. Panarin, E. F., M. V. Solovskii, N. A. Zaikina and G. E. Afinogenov. 1985. *Makromol. Chem. Suppl.*, 9:25.
26. Ikeda, T., H. Yamaguchi and S. Tazuke. 1984. *Antimicrob. Agents Chemother.*, 26:139.
27. Ikeda, T., H. Hirayama, H. Yamaguchi, S. Tazuke and M. Watanabe. 1986. *Antimicrob. Agents Chemother.*, 30:132.
28. Ikeda, T., S. Tazuke and Y. Suzuki. 1984. *Makromol. Chem.*, 185:869.
29. Ikeda, T., H. Hirayama, H. Yamaguchi and S. Tazuke. 1986. *Makromol. Chem.*, 187:333.
30. Hugo, W. B. and A. D. Russell, eds. 1980. *Pharmaceutical Microbiology*. Oxford: Blackwell, p. 3.
31. Costerton, J. W. and K.-J. Cheng. 1975. *J. Antimicrob. Chemother.*, 1:363.
32. Hamilton-Miller, J. M. T. 1965. *Biochem. Biophys. Res. Commun.*, 20:688.
33. White, D. A., W. J. Lennarz and C. A. Schnaitman. 1972. *J. Bacteriol.*, 109:686.
34. Costerton, J. W., J. M. Ingram and K.-J. Cheng. 1974. *Bact. Rev.*, 38:87.
35. Katchalsky, A. 1964. *Biophys. J.*, 4:9.
36. Hugo, W. B. and A. R. Longworth. 1964. *J. Pharmac.*, 16:655.
37. Salton, M. R. 1951. *J. Gen. Microbiol.*, 5:391.
38. Lambert, P. A. and S. M. Hammond. 1973. *Biochem. Biophys. Res. Commun.*, 54:796.
39. Hugo, W. B. and A. R. Longworth. 1966. *J. Pharmac.*, 18:569.
40. Hawker, L. E. and A. H. Linton, eds. 1979. *Microorganisms*. London: Edward Arnold, Chapter 8.
41. Chapman, D. 1975. *Quart. Rev. Biophys.*, 8:185. Papahajopoulos, D., W. J. Vail, W. A. Pangborn and G. Poste. 1976. *Biochim. Biophys. Acta*, 448:265. Cullis, P. R. and B. de Kruijff. 1978. *Biochim. Biophys. Acta*, 513:31. There are many other references.
42. Hauser, H., K. Howell and M. C. Phillips. 1977. *FEBS Lett.*, 80:355.
43. Rack, J. L. and J. F. Tocanne. 1982. *FEBS Lett.*, 143:171.
44. Sixl, F. and H.-J. Galla. 1981. *Biochim. Biophys. Acta*, 643:626.
45. Eliasz, A. W., D. Chapman and D. F. Ewing. 1976. *Biochim. Biophys. Acta*, 448:220.
46. Tirrell, D. A. and P. M. Boyd. 1981. *Makromol. Chem. Rapid Commun.*, 2:193.
47. Takigawa, D. Y. and D. A. Tirrell. 1985. *Macromolecules*, 18:338.
48. Tirrell, D. A., A. B. Turek, D. A. Wilkinson and T. J. MacIntruch. 1985. *Macromolecules*, 18:1512.
49. Turek, A. B. and D. A. Tirrell. 1986. *J. Bioact. Comp. Polym.*, 1:309.
50. Ikeda, T., S. Tazuke and M. Watanabe. 1983. *Biochim. Biophys. Acta.*, 735:380.
51. Ikeda, T., A. Ledwith, C. H. Bamford and R. A. Hann. 1984. *Biochim. Biophys. Acta*, 769:57.
52. Galla, H.-J. and E. Sackmann. 1975. *Biochim. Biophys. Acta*, 401:509.
53. Birrell, G. B. and O. H. Griffith. 1976. *Biochemistry*, 15:2925.
54. Boggs, J. M., M. A. Moscarello and D. Papahadjopoulos. 1977. *Biochemistry*, 16:5420.
55. Boggs, J. M., D. D. Wood, M. A. Moscarello and D. Papahadjopoulos. 1977. *Biochemistry*, 16:2325.
56. Ikeda, T., R. Lee, H. Yamaguchi and S. Tazuke. 1990. *Biochim. Biophys. Acta*, 1021:56.
57. Ikeda, T., H. Yamaguchi and S. Tazuke. 1990. *Biochim. Biophys. Acta*, 1026:116.
58. Schaefer, G. 1976. *Biochem. Pharmacol.*, 25:2015.
59. Eisenhans, B., R. Blume, B. Lembeke and W. E. Caspary. 1983. *Biochem. Biophys. Acta*, 727:135.
60. Isquith, A. J., E. A. Abbott and P. A. Walters. 1972. *Appl. Microbiol.*, 24:859.
61. Walters, P. A., E. A. Abbott and A. J. Isquith. 1973. *Appl. Microbiol.*, 25:253.
62. Nakagawa, Y., H. Hayashi, T. Tawaratani, H. Kourai, T. Horie and I. Shibasaki. 1984. *Appl. Environ. Microbiol.*, 47:513.
63. Yamizori, O. 1983. *J. Antibact. Antifung. Agents*, 11:76.
64. Walfish, I. H. and G. E. Janauer. 1979. *Water, Air and Soil Pollution*, 12:477.

65. Gerba, C. P., G. E. Ianauer and M. Costello. 1984. *Water Res.*, 18:17.
66. Nakagawa, Y., Y. Yamano, T. Tawaratani, H. Kourai, T. Horie and I. Shibasaki. 1982. *Appl. Environ. Microbiol.*, 43:1041.
67. Nakagawa, Y., T. Tawaratani, H. Kourai, T. Horie and I. Shibasaki. 1984. *Appl. Environ. Microbiol.*, 47:88.
68. Kawabata, N. T. Hayashi and T. Matsumoto. 1983. *Appl. Environ. Microbiol.*, 46:203.
69. Kawabata, N., T. Hashizume and T. Matsumoto. 1986. *Agric. Biol. Chem.*, 50:1551.
70. Kawabata, N. and Kako Kobunshi. 1985. 34:583.
71. Rosenberg, M., D. Gutnick and E. Rosenberg. 1980. *FEMS Microbiol. Lett.*, 9:29.
72. Rosenberg, M., S. Rottem and E. Rosenberg. 1982. *FEMS Microbiol. Lett.*, 13:167.
73. Nakagawa Y., T. Tawaratani and I. Shibasaki. 1979. *J. Antibact. Antifung. Agents*, 7:T511.
74. Nakagawa, Y., Y. Inoue, T. Tawaratani and I. Shibasaki. 1979. *J. Antibact. Antifung. Agents*, 7:T515.
75. Nakagawa, Y., N. Doki, T. Tawaratani and I. Shibasaki. 1983. *J. Antibact. Antifung. Agents*, 11:263.
76. Ikeda, T., H. Yamaguchi and S. Tazuke. 1986. *J. Bioact. Comp. Polym.*, 1:162.
77. Ikeda, T., H. Yamaguchi and S. Tazuke. 1986. *J. Bioact. Comp. Polym.*, 1:301.
78. Ackert, W. B., R. L. Camp, W. L. Wheelwright and J. S. Byck. 1975. *J. Biomed. Mater. Res.*, 9:55.

COVALENT ATTACHMENT OF HYDROPHILIC GROUPS ONTO THE SURFACE OF LOW DENSITY POLYETHYLENE

BY

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Ali's MS Thesis

The author dedicates this thesis to his parents
who supplied endless support and encouragement.

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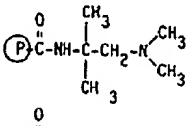
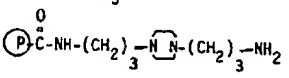
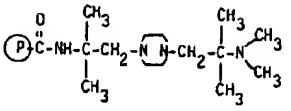
30	FT-IR/ATR Spectrum for 	60
31	FT-IR/ATR Spectrum for 	60
32	FT-IR/ATR Spectrum for 	61

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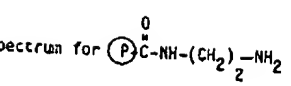
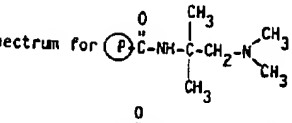
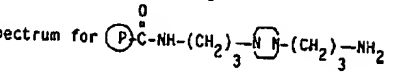
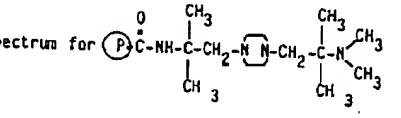
33	XPS Spectrum for 	62
34	XPS Spectrum for 	62
35	XPS Spectrum for 	63
36	XPS Spectrum for 	63
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ABBREVIATIONS

DMAEMA	Dimethylaminoethyl methacrylate
Q-DMAEMA	Trimethyl aminoethyl methacrylate ammonium iodide
AM	Acrylamide
LDPE	Low density polyethylene
Ce^{4+}	Ceric ammonium nitrate
XPS	X-ray photoelectron spectroscopy (also known as ESCA)
FT-IR/ATR	Fourier Transform infra-red attenuated total reflectance
SEM	Scanning electron microscopy
NMR	Nuclear magnetic resonance

Abstract of Thesis Presented to the Graduate School of the
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COVALENT ATTACHMENT OF HYDROPHILIC GROUPS ONTO THE SURFACE OF LOW DENSITY POLYETHYLENE

By

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May 1986

Chairman: Christopher D. Batich
Major Department: Materials Science and Engineering

Several methods of chemically modifying the surface of low density polyethylene (LDPE) were examined. A chromic acid oxidation similar to a published description was used to form a highly carboxylic acid surface. This surface was then either reacted with polyamines to form amido-amine structures or reduced to an alcohol with diborane.

The amido-amine structure was considered for a potential alkylation to synthesize a quaternary ammonium group on the surface. The resulting ammonium bearing surface should have an important application for antibacterial activity. This last step has not yet been carried out successfully. It is believed that steric effect emerging from the solid state nature of the substrate could inhibit the reaction.

The hydroxylated surface was used as a substrate to graft polymerize acrylamide using Ce^{4+} as an initiator. This initiator is

known to generate transient radicals with alcohols in solution inducing polymerization if a vinyl monomer is present. In this study, a substantial grafting of acrylamide onto solid LDPE was obtained.

The last method was the plasma-induced graft copolymerization of vinyl monomers bearing a quaternary ammonium group. Trimethyl amino ethyl methacrylate ammonium iodide synthesized for this purpose was graft copolymerized with acrylamide in water onto a plasma-activated LDPE surface. The XPS and FT-IR/ATR data brought evidence that this grafting was carried out. Although the yields are still low, this novel method proved to be simple and awaits further development regarding the optimization of the reaction conditions.

Characterization methods involved contact angle measurements, X-ray photoelectron spectroscopy, Fourier Transform infra-red attenuated total reflectance, scanning electron microscopy, dye uptake, and NMR.

Dr. C. D. Batich, Chairman

1 INTRODUCTION

Surface structure and composition play a major role in defining many of the physical properties and ultimate uses of solid organic polymers. In engineering and biomedical applications, features such as wetting, weathering, adhesion, friction, electrostatic charging, permeation, biocompatibility, and bacterial fouling are largely dependent on surface properties.

Surface properties determine the value in use of many solid materials. Such properties are as critical as bulk parameters such as strength, elongation, Young's modulus, etc. Furthermore, full utilization of bulk properties is often supported by surface characteristics which are mainly dependent on surface energetics (e.g. surface tension). This is particularly the case of fibers, films, membranes or other cases where the surface area/volume ratio is large.

In this research we performed a series of modifications on the surface of LDPE (low density polyethylene). LDPE is the most widely used polymeric material because of its good mechanical properties, chemical inertness, low cost, and ready availability. However, its hydrophobicity represents a disadvantage in a variety of applications.

From a technical standpoint it is still difficult to obtain polymers with surfaces having well defined functionality. Polymer

molecules in the vicinity of the surface or interface show different thermodynamic behavior than the molecules in the bulk due to the different interfacial environment (1). Surface thermodynamics requires that, where possible, rearrangements of molecules in the surface occurs such that the surface which is presented to air has the minimum free energy (2). Therefore, it is difficult to construct and test hypotheses relating the molecular structure of a polymer surface to its macroscopic surface properties. Consequently, a variety of methods have been developed for functionalizing polymer surfaces to give materials whose structure is well understood at the molecular level. The stability of such high free energy surfaces on bulk phases with the lower surface tensions is sometimes low.

D. Dwight (3) has reviewed the techniques for surface modification of polymers. Among the methods available, the wet chemical treatment, based on chromic acid oxidation, has been pointed out to have the advantage of changing both the chemical and the physical nature of the polymer surface (Figure 1).

J. R. Rasmussen et al. (4,5) have considerably improved the chromic acid oxidation method by optimizing the experimental parameters in order to get a maximum density of carboxylic groups on the surface. In this study, Rasmussen's methodology has been used to polarize our LDPE film. The carboxylic groups generated were then covalently coupled with a series of polyamines using dicyclohexylcarbodiimide (DCC) as a dehydrating agent (Figure 2). The coupling led to an amido-amine group covalently attached to the surface. The products were well characterized by FT-IR/ATR and XPS.

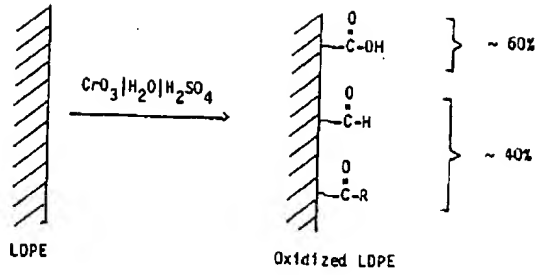

Figure 1. Chromic acid oxidation scheme and functional group distribution. Source: Ref. 4

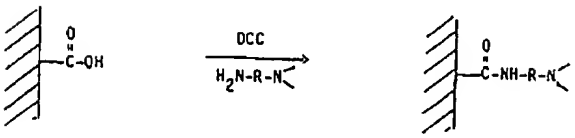

Figure 2. Coupling reaction of a carboxylic acid and a diamine.

A long term objective of this research was to develop a synthetic procedure to covalently attach a quaternary ammonium salt and other hydrophilic groups on the surface for antibacterial activity and improvement of cell adhesion, respectivaly.

Quaternary ammonium salts are well known for their antibacterial activity (6,7).

In this study different approaches to quaternize the LDPE surface were used. First, the direct alkylation of the terminal amino group was attempted (Figure 2). The reactivity of the amine seems drastically reduced by the two-dimensional aspect of the surface which has steric limitations as well as interface forces. The second approach was the direct grafting of a quaternary ammonium-bearing vinyl monomer by using plasma-induced graft polymerization. The FT-IR/ATR and XPS analyses showed that the second approach was a better alternative.

On the other hand, acrylamide was grafted onto LDPE surface. The grafting was carried out by first reducing the carboxyl groups on the oxidized LDPE to alcohols, then using ceric ammonium nitrate as a redox initiator system, induced graft polymerization of acrylamide. Such hydrophilic surface should have a variety of biological consequences such as cell adhesion, bacterial toxicity or modified thrombogenicity. Such biological testing has, in general, not been attempted here and awaits further research.

2 BACKGROUND

2.1 Importance of Interfacial Phenomena on Solid Surfaces

The surface energy and interfacial parameters and their interrelationships with biocompatibility and also bacterial adhesion to synthetic polymers have been of high interest in recent years (8). However, because of the complexity and interdependence of the many parameters involved, no individual theory provides a satisfactory explanation of the biological tolerance of the solid polymers currently in use (9).

Chemical inertness was formerly the primary restriction which guided the development of biomaterials. However, once exposed to the physiological medium, the interfacial properties of even chemically inert surfaces may be modified by interaction with various microorganisms which modify the surface by chemical changes, deposition of protein molecules or attachment of living or dead cells.

Substantial evidence suggests that surface characteristics such as polarity or chemical functional groups available for reaction, largely influence interfacial phenomena in biological systems (10-14).

R. E. Baier et al. have reviewed the surface chemistry and physics of biological adhesion (15). K. C. Marshall et al. have presented arguments and evidence which emphasize the possible importance of

electrical charge and dipole interactions in attracting bacteria to surfaces (16,17). It is also strongly believed that most bacteria show a preference to attach on hydrophobic surfaces (12).

Although the mechanisms of the biological interfacial phenomena are still poorly understood and often controversial because of the complexity of biological systems, surface properties of the solid polymer are emphasized as key parameters (12).

2.2 Surface Modification Techniques

The surface modification of polymer films is an area of considerable technological and industrial importance. Although many polymer materials could be up-graded if a satisfactory modification technique were available, relatively minor efforts have been devoted to this field in comparison with the tremendous research activities directed to the development of new polymers. One reason for this was the ambiguity of methods for studying surface structure and surface properties. However, in recent years, a considerable progress has been made in the investigation of surface treatments of polymers. This was made possible by a dramatic increase in the sophistication of the spectroscopic methods for surface analysis particularly XPS and FT-IR.

In the particular case of polyolefins, a variety of chemical treatments have been described to change the characteristics of polymer surfaces (3). Such treatments fall into two general categories depending whether a wet chemical process is involved or whether the interaction occurs at the gas-solid interface as would be the case in a

flame, electrical discharge treatment, γ-irradiation or glow discharge treatments, all of which appear to result in an enhanced degree of surface oxidation.

In this study several methods for surface modification of LDPE were considered.

2.2.1 Chemical Oxidation

The chemical modification of the LDPE surface has been particularly the subject of extensive research (4,5,18,19). Most procedures involve reactive species capable of diffusing some distance into the bulk of LDPE, and, as a consequence, introduce functional groups both onto the surface and into the bulk polymer. Wet chemical treatment provides a convenient means of functionalizing polymer films.

B. Gatoire et al. have studied the surface oxidation of polyethylene by exposure to $KClO_3/H_2SO_4$ (18). The main interest of this study was the use of ESR (electron spin resonance) spectroscopy and $^{45}Ca^{2+}$ trace adsorption techniques to study the oxidized surface. Mn^{2+} which bonds specifically to the carbonyls generated on the surface, was used as an ESR probe. Quantitative information regarding the density of polar sites on the surface was obtained from the intensity of the ESR signal. Also, information concerning the macromolecular structure at the polymer interface were obtained. Spin-spin interaction between Mn^{2+} neighbors and a high density of hydrogen bonds produced weaker and wider ESR signals.

J. C. Ericksson et al. have oxidized the surface of polyethylene by using three different types of oxidizing agents: $KMnO_4$, $KClO_3$, and $K_2Cr_2O_7$, each dissolved in H_2SO_4 (19). Although all three oxidizing agents lead to highly hydrophilic surfaces, their XPS investigation showed some major differences regarding the extent of oxidation and also the nature of the polar groups generated on the surface. The O1s/C1s intensity ratios obtained from XPS show that KM_nO_4/H_2SO_4 is a more powerful oxidant than $KClO_3/H_2SO_4$ which, in turn, is somewhat more powerful than $K_2Cr_2O_7/H_2SO_4$. Then using chemical tagging and XPS, the nature of the particular polar groups was determined for each oxidizing agent. It was found that KM_nO_4/H_2SO_4 generated a high content of unstable hydroperoxide groups. No evidence for these groups was found for the other oxidizing agents. With $KClO_3/H_2SO_4$ some hydrocarbon chlorination was detected by XPS. Comparison of the carbon 1s peaks obtained with each oxidant showed that $K_2Cr_2O_7/H_2SO_4$ treatment generated the higher content of $-\overset{O}{\overset{\|}{C}}-$, $-\overset{O}{\overset{\|}{C}}-H$ and $-\overset{O}{\overset{\bullet}{C}}-OH$ groups.

Earlier, in 1977, J. R. Rasmussen et al. (4,5) developed similar but more laborious methodology of oxidation of LDPE based also on chromic acid oxidation. Of the chromic acid solutions tested, $CrO_3/H_2O/H_2SO_4$ in the ratio 3:4:3 by weight produced a functionalized LDPE film with the fewest non carbonyl IR/ATR absorptions. Also, a reaction temperature of 72°C and a reaction time of 5 minutes were found to cause the least damage to the bulk polymer and to generate the highest carboxylic acid groups on the surface. In this work, useful procedures for attaching derivatives covalently to the carboxyl groups

were developed as well as optical characterization methods. For instance, fluorescence spectroscopy was shown to be a useful analytical tool to estimate, with high accuracy, the numbers of functional groups of appropriate derivatives attached to the functionalized LDPE surface.

In our study, we mainly adopted Rasmussen's methodology to generate carboxylic groups on the LDPE surface. However, we used different subsequent chemical reactions and also characterized our products with analytical techniques such as XPS and FT-IR/ATR.

2.2.2 Amido-Amine Modified LDPE

Carboxylic groups generated on the LDPE surface represent versatile organic functional groups for subsequent derivatization. A classical method of forming an amide from a carboxyl group is through the acid chloride derivative (Figure 3). Although this method gives good yields, it has the disadvantage of hydrocarbon chlorination as pointed out by R. G. Nuzzo and G. Smolinsky (20). Apparently some $SOCl_2$ and SO_2 (by-product) diffuse into the bulk where they remain strongly incorporated even after a soxhlet extraction with 2-propanol. A nitrogen purge of the reaction medium did not help to remove the SO_2 by-product.

In our study, instead we used DCC as a dehydrating agent to couple the carboxylic group to several polyamines (Figure 2). The reaction of carboxylic acid with carbodiimides is known as a very important precursor for the synthesis of peptides (21). Today, DCC is still the most used reagent in that field (22). The yields are as good as the acid chloride method but offers the advantages of easier handling and no side

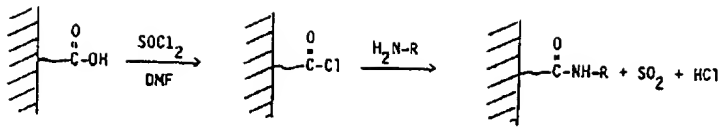

Figure 3. Scheme of the amide formation through the acyl chloride derivative.

reactions. We were able to graft several polyamines on the oxidized LDPE and the results were reproducible.

2.2.3 Cation-Bearing Polymers

2.2.3.1 Importance of cation-bearing polymers

The tremendous utility of polyelectrolyte polymers has led to a vigorous field of industrial research over the last 25 years. Some of the most versatile and useful types of polyelectrolytes are the cationics or quaternary ammonium bearing polymers. G. B. Butler and M. F. Hoover reviewed extensively the ion-containing polymers (23). They emphasized their utility in solving solid/liquid separation problems related to water pollution and also their extensive use in paper manufacturing, textile finishing, oil production, plastics, coatings, biomedical applications, etc.

While significant work has appeared on water soluble polyelectrolytes, papers and technical information on the solid organic polymers as carriers of ammonium groups are scarce.

2.2.3.2 Examples of quaternized surfaces

A. J. Isquit et al. (6) reported on the silanol covalent bonding of quaternary ammonium salts to a variety of activated surfaces (Figure 4). The surfaces treated are usually higly polarized such as glass, synthetic, and natural fibers. An outgrowth of this work led to commercialization of a surface treatment method called "Bioguard" by Dow Corning Corporation.

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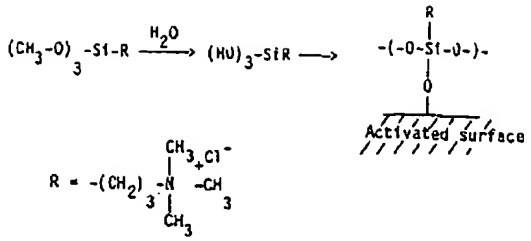
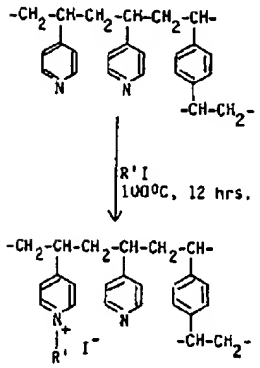
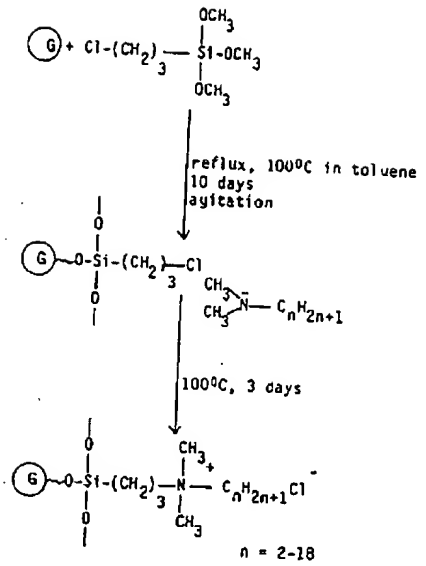


Figure 4. Quarternization scheme of an activated surface.
Source: Ref. 6



R' = C_8-C_{18} alkyl

Figure 5. Quaternization scheme of 4-vinyl pyridine-divinylbenzene copolymers in solution.
Source: Ref. 7



G = Porous glass

Figure 6. Quaternization scheme of porous glass.
Source: Ref. 24

Investigation by Y. Nakagawa et al. (7) on insolubilization of quaternary ammonium salts on cation exchange resin was based on alkylation of 4-vinylpyridine-divinylbenzene copolymers (Figure 5). The quaternization was carried out under severe conditions (100°C, reflux for 12 hours with alkyl iodides). In another publication (24), Y. Nakagawa reported on quaternization of porous glass. The nature of the surfaces synthesized were similar to Isquit's (6), however, the synthetic route was different (Figure 6). It can be seen from Figure 6 that the reaction conditions used were extremely severe.

2.2.3.3 Quaternization of LDPE

The particularly severe reactions conditions used in the above examples of quarternized surfaces are not suitable for the LDPE used in this study. Also, even after chromic acid oxidation, LDPE contains much fewer active sites than glass for instance. Thus, none of the above procedures can be applied to our substrate. In addition, no literature is available concerning the quaternization of solid state LDPE. Therefore, we examined other methods with milder reaction conditions.

The first method, as already mentioned, consisted of alkylating the terminal amine group on derivatized LDPE (Figure 2). Although no such reaction is reported in the literature we believe that it could be an interesting route provided that optimal reaction conditions be found. In solution quaternization of amines is easily carried out.

The second method consisted on plasma-induced graft polymerization of an ammonium-bearing vinyl monomer that we synthesized. The conventional plasma approach of surface modification of solid polymers

is to simply expose the surface to a gas phase monomer under plasma excitation. However, this method suffers some serious disadvantages such as the complexity of the mechanisms involved and also the wide variety of reactive species formed (25). Conventional plasma grafting is non specific in nature because the stoichiometry and functional group distribution in polymerized materials are not related in a simple manner to those of the monomer (26).

On the other hand, there is a new approach described to use plasma for initiation of polymerization of liquid monomers (26) and also inducing graft polymerization on solid surfaces (27).

Y. Osada and Y. Iriyama (27) reported on the plasma-initiated graft polymerization of water soluble vinyl monomers onto hydrophobic films such as LDPE, PP, and PET. The polymers obtained are non cross linked and have extremely high molecular weights. Monomers such as acrylic acid, acrylamide and hydroxyethyl methacrylate have been effectively graft polymerized. However, the quaternized form of dimethyl amino ethyl methacrylate (Q-DMAEMA) has not been described and hence was examined here, using a similar procedure.

While the results of Y. Osada et al. (26) seemed to indicate that plasma was suitable for graft polymerization, questions regarding the kinetics and the mechanisms involved remain controversial since some aspects of a conventional radical polymerization are often not obeyed (27).

D. R. Johnson et al. studied the mechanism and kinetics of plasma-initiated polymerization of methyl methacrylate (28). The experimental

set up was as follows: The liquid monomer was degassed, sealed in a thin-walled ampule and then frozen in liquid nitrogen. The ampule was then inserted between a pair of parallel-plate electrodes connected to an r.f. (radio frequency) plasma generator. The ampule was permitted to warm up until droplets of liquid monomer appeared. A glow discharge was then initiated in the vapor phase above the partially frozen monomer. The results of this study provided evidence that plasma-initiated polymerization of methyl methacrylate proceeds by chain propagation via a free-radical mechanism.

On the other hand, Y. Osada et al. raised several questions when only the radical mechanism is considered. First, it was noticed that plasma-iniated polymerization was selective towards the monomer species. Alkyl acrylates, styrene, acrylonitrile, N-vinyl pyrolidone and other vinyl monomers are not susceptible to polymerization. Second, polymerization proceeds for a long period of time (up to 7 days to obtain an 80% conversion). Third, there is evidence that the polymerization process is strongly affected by solvents. The ratio of the rates of polymerization in water and DMF was as large as 4000. However, it is known that the rate of radical polymerization is not significantly influenced by solvents in principle. Y. Osada et al. (29) who made the above observations did not draw a final conclusion concerning the polymerization mechanism but did envision a mechanism for the initiation process (Figure 7) in terms of the interaction between active species diffused from the gaseous plasma and liquid monomers. The possibility of solvation of ionic species was also proposed. In

Gas phase	$M_g + e \longrightarrow M_g^*(+e)$		(1)
Gas-liquid interphase	$M_g^* \longrightarrow M_l^*$		(2)
Solvation of reactive species	$M_l^* + S \longrightarrow (M_l^*)_s$		(3)
Initiation	$(M_l^*)_s + M_1 \longrightarrow (M_l^*)_s$		(4)
	or $(M_l^*)_s + M_1 \longrightarrow M_1^*$		(5)
Propagation	$(M_l^*)_s + M_2 \longrightarrow (M_2^*)_s$		(6)
	or $M_1^* + M_2 \longrightarrow M_2^*$		(7)

Figure 7. Mechanism scheme for formation of active species in the initiation step of plasma-induced polymerization. Source: Ref. 29

Figure 7. M_g^* represents the species formed as a result of the direct interaction of the monomer vapor with energetic electrons in the gas phase. Some of them can then diffuse into the liquid phase and become M_l^* or $(M_l^*)_s$ after solvation, which can then induce polymerization. As already mentioned, the reactivity of the reactive species $(M_l^*)_s$ is strongly influenced by solvent. Thus, polymerization is closely associated with the activity of the solvated species.

Thus far, the only mechanisms reported in the literature involve plasma-induced polymerization of liquid monomers. No mechanism, to our knowledge, concerning the plasma-induced graft polymerization of liquid monomers to activated solid substrates is reported.

2.2.4 Graft Polymerization of Acrylamide onto LDPE by Ce^{4+} Initiation

It is known that in the presence of organic reducing agents such as alcohols, Ce^{4+} is an effective redox system producing Ce^{3+} and transient free radical species. If a vinyl monomer is present, the free radical initiates polymerization (30).

S. B. Vitta et al. (31) reported on the grafting of acrylic acid and methacrylic acid onto solid state cellulose via ceric ion initiation. It has been found that in relatively short times (2-5 hours) grafting up to 200% was obtained.

In this study, it has been possible to graft polymerize acrylamide onto LDPE by using the Ce^{4+} initiation. Our rational was based on the generation of hydroxyl groups on the LDPE surface. This was possible by

reducing with diborane the carbonyls present on the oxidized LDPE surface. XPS and FT-IR/ATR data proved that a substantial grafting was obtained.

3 MATERIALS AND METHODS

3.1 Materials

Polyethylene film used, in this study, was a low density, 4 mil, commercial grade prepared from Northern Petrochemical 940 resin (0.918 density, 0.25 melt index). The film was prepared and provided by Central States Diversified, Inc., Palatka (Florida). Chromium trioxide, dicyclohexylcarbodiimide, methyl iodide, ethyl iodide, acrylamide (recrystallized twice from chloroform), ceric ammonium nitrate and ethylene diamine were reagent grade from Fisher Scientific Co. 1-dimethyl-amino-2-amino-2-methylpropane and 1,4-Bis (2-amino-2-methylpropyliperazine) were provided by Angus Chemical Company. Bis (aminopropyl) piperazine was provided by Texaco Chemical Co. Dimethylamino-ethyl methacrylate, distilled under vacuum (13 mm Hg) at 76°C, was from Polysciences. Diborane (1M in THF) and trifluoroacetic anhydide were reagent grades from Aldrich.

3.2 Methods

3.2.1 Sample Preparation

In order to remove any surface contamination, LDPE films precut to the appropriate size (usually 2x5 cm^2) were enclosed in filter paper thimbles and extracted in a soxhlet apparatus with 2-propanol for 18

hours. After extraction, the films were removed from the envelopes, and dried in vacuum (about 10^{-2} torr) at 50°C for 4 hours. Alternatively, the samples were sonicated in methylene chloride for 15 minutes and the results were similar.

3.2.2 Oxidation

The procedure employed was similar to that reported in the literature (4,5). Reactions performed on LDPE film were normally with 2x5 cm^2 pieces. The oxidizing agent was a mixture of chromium trioxide, water, and concentrated sulfuric acid in a 3:4:3 ratio by weight. As already mentioned, J. R. Rasmussen et al. optimized the reaction conditions for this oxidation (4,5). It was found that reaction time of 5 minutes and a temperature of 72°C gave the best results regarding the extent of oxidation, the type of oxidation product and causing the minimuim damage to the bulk. Typically 50 ml of chromic acid solution was used. Agitation was provided by manually swirling the beaker because magnetic stirring often resulted in abrasion of the film. After the reaction time, the sample is removed by Teflon coated forceps and put, for 15 minutes, in a 70% HNO_3 solution at 50°C in order to remove any inorganic impurity on the surface. Then the sample is thoroughly washed with deionized water at 50°C and finally rinsed with acetone and dried in vacuum (~ 10^{-2} torr) for 4 hours.

The extent of oxidation was determined by monitoring the reaction time and maintaining all other parameters constant. The extent of oxidation was characterized by contact angle measurements and XPS

analysis. FT-IR/ATR and XPS data show that the oxidized surfaces contain predominantly $-\overset{O}{\overset{\|}{C}}-OH$, $-\overset{O}{\overset{\|}{C}}-$, and $-\overset{O}{\overset{\|}{C}}-H$ groups. (Characterization details are in Section 4.1.1.)

3.2.3 Amido-Amine Modified LDPE

Further modification of the oxidized LDPE surface is possible by coupling the carboxyl group with an amine. Several synthetic methods exist. The most classical route consists of converting the carboxyl group to the corresponding acyl chloride by treatment with thionyl chloride. As mentioned earlier, this reaction suffers from some side reactions. A second alternative was the use of DCC (dicyclohexyl-carbodiimide) as a dehydrating agent to couple $-\overset{O}{\overset{\|}{C}}-OH$ and $-NH_2$ groups. Carbodiimides have been particularly valuable in the synthesis of peptides and of nucleotides (22,32) because of their ability of affecting acylations under mild conditions and ease of use. Other advantages will be discussed later.

The DCC procedure consists of putting the oxidized LDPE film in 50 ml of DMF (dimethyl-formamide), and then cooling in an ice-bath to 0°C. Then 200 mg of DCC predissolved in 10 cc DMF were added. After 15 minutes, an excess of diamine is added. The reaction mixture is kept at 0°C for 30 minutes, then is allowed to proceed for 6-8 hours at room temperature (32). The film was then removed, washed with acetone, extracted with 2-propanol for 2 hours and finally dried in vacuum for 2 hours. The samples are characterized with XPS and FT-IR/ATR.

3.2.4 Quaternization of the Amido-Amine Modified LDPE

The amido-amine group shown in Figure 2 should be suitable for subsequent quaternization with alkyl halides. Our strategy was based on the principles of alkylation of primary, secondary and tertiary amines in solution (33,34). Typically the amido-amine modified LDPE film was put in 50 ml of DMF, then an alkyl halide (methyl or ethyl iodide) was added in large excess but gradually over a period of 8 hours. The reaction was carried out at room temperature, under N_2 atmosphere and in the dark because of the instability of the alkyl iodides to light and oxygen. When the terminal amino group is primary or secondary, a proton scavenger (usually diisopropyl ethyl amine or tri-n-butyl amine) is added in order to avoid the protonation of the grafted amine group.

This reaction was unsuccessful in all cases tried on solid surfaces.

3.2.5 Diborane Reduction of Oxidized LDPE

In order to generate the alcohol functionality on the surface, the oxidized LDPE film was covered, under N_2 atmosphere, with 1.0M diborane in THF (tetrahydrofuran) and allowed to stand for 18 hours at room temperature (35). The film was then removed and washed sequentially with THF (twice), methanol, 4N sulfuric acid (50°C, twice), water (twice) and acetone. The FT-IR/ATR analysis shows the almost complete disappearance of the carbonyl peak (details are in Section 4.1.4).

3.2.6 Derivatization of the Surface Alcohol

Samples of diborane-reduced LDPE were incubated with 3 ml of trifluoroacetic anhydride in 50 ml of etheral solution and let to react for 6 hours (4,35). Then the film is washed thoroughly with ethyl ether, acetone, and then dried in vacuum. The FT-IR/ATR spectrum shows a strong ester peak at 1784 cm^{-1} (Figure 52). The ester is shifted to higher wavenumber because of the electron withdrawing effect of the fluorine.

3.2.7 Graft Polymerization of Acrylamide onto LDPE by Ce^{4+} Initiation

The alcohol-modified LDPE is put in 40 ml of distilled water which was degased for 20 minutes by N_2 purge. Then an appropriate amount of acrylamide (0.5 mol/l) and Ce^{4+} ion ($5x10^{-3}$ mol/l) were added. After purging with N_2 for another 10 minutes, the reaction is carried out at room temperature overnight. Several extractions in a soxhlet with CH_3OH, DMF and water were used in order to remove any residual monomer and physically adsorbed homopolymer. The products were characterized with FT-IR/ATR and XPS.

3.2.8 Plasma-Induced Graft Polymerization of Vinyl Monomers onto LDPE

The plasma-induced graft polymerization or copolymerization were carried out by the direct exposure of the plasma treated film to a liquid monomer as described by D. R. Johnson et al. (28). The r.f. (radio frequency) plasma reactor used was a bell-jar-like model normally used for deposition or polymerization of gazeous monomers. In our

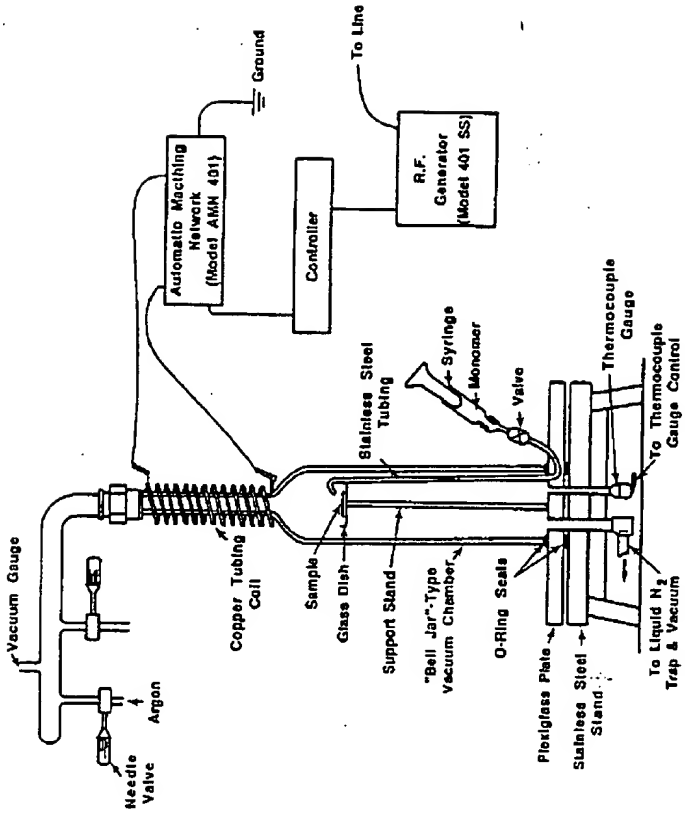


Figure 8. Schematic drawing of a modified R.F. plasma apparatus (R.F. Plasma Products Inc., HFS 401 S).

experiment, we modified the apparatus so that liquid monomers could be introduced, for graft polymerization onto the activated substrate, under vacuum. The details of the experimental plasma set up are shown in Figure 8.

The film to be grafted was placed horizontally in a glass plate inside the r.f. plasma reactor. A glow plasma was then generated under the following operating parameters: time = 90 seconds, power = 25 watts, vacuum = 100 μm Hg, gas = argon. The aqueous solution of monomer which had been previously degased was injected into the reactor and on the plasma-treated film as shown in Figure 8. The monomer is left to post-polymerize at room temperature for 12 hours.

3.2.9 Characterization

3.2.9.1 FT-IR/ATR

Fourier-transform infra-red attentuated total reflectance spectrometry is one of the most frequently used tools for surface characerization of polymer materials (36) and has been the most popular means of gaining surface sensitivity for optical spectroscopic techniques (13).

The advantages of ATR as a surface analytical method is its ability to give much information on a surface such as chemical composition and chemical structure orientation and conformation (37), crystallinity (37,25), hydrogen bonding, etc. The capability of ATR spectroscopy for surface analysis is attributed to the penetrating nature of the light on total reflection from the internal reflection element (IRE)/sample interface into the sample (36).

ATR has had a relatively long history of use in the biomaterials field (2,38). The ATR technique has also been used in a number of instances to study surface modification of polymer films (4,5,38,10). An NIH sponsored center for FT-IR analysis of biomaterials exists at Battelle Laboratories (Columbus, Ohio).

However, one of the major requirement for ATR is that a "good" contact between the IRE and sample must be attained. A bad contact between the sample and the IRE results in noisy and distorted spectra with non horizontal baselines.

In this study, FT-IR/ATR spectra were collected with a Nicolet 60 SX spectrometer equipped with an MCT detector. The ATR stage was a Wilks model 50, which is capable of collecting spectra at 30°, 45° and 60° angle of incidence (with respect to surface normal). The two samples (1x5 cm^2) were pressed against a KRS-5 crystal which was face cut at 45° for analysis. In order to maintain the same quality of contact, the samples were clamped against the IRE with a torque wrench at 5 in. lbs. Typically 500 spectra were averaged for the ATR experiments. All spectra were referenced to the base KRS-5 crystal, at the same angle. All spectral manipulations were done with standard Nicolet software.

3.2.9.2 XPS

X-ray photoelectron spectroscopy (XPS), also known as electron spectroscopy for chemical analysis (ESCA), has already shown itself to be a valuable technique for studying the structure of polymers (39,40). This technique is a relatively new analytical tool which

provides high information content on chemical structure and bonding. Of all the presently available instrumental techniques for surface anlaysis, XPS is generally regarded as being among the most quantitative, the most readily interpretable, and the most informative with regard to chemical information (1). For these reasons, XPS has found a large application for the analysis of biopolymers (41). An NIH center for XPS analysis of biomaterials exists at the University of Washington at Seattle, Washington.

The basic principle of XPS is the photoelectric effect. Figure 9 represents schematically the interaction of a photon with an atomic orbital electron.

Given that the photon energy is greater than the binding energy of the electron in the atom, the electron is then ejected from the atom with a kinetic energy approximately equal to the difference between the photon energy and the binding energy. The basic equation for XPS is:

$$E_b \cong h\nu - E_k$$

where E_b is the electron binding energy, E_k is the electron kinetic energy measured by the instrument, and $h\nu$ is the photon energy (h is Planck's constant and ν is the x-ray frequency). All energies are expressed in electron volts (ev). Knowing the binding energy, one can identify the atom.

The basic advantages and disadvantages of XPS are listed as follows:

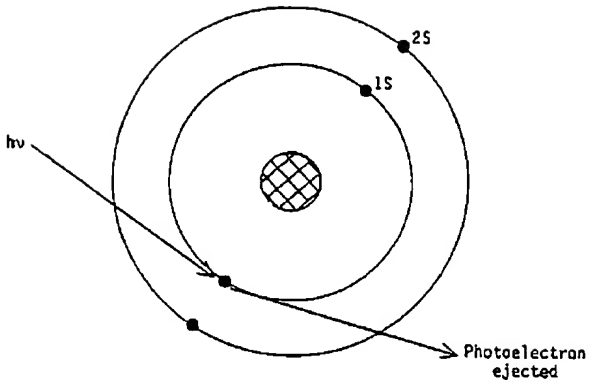


Figure 9. Schematic view of the interaction of an x-ray photon with an atomic orbital.

Advantages:

Nondestructive

Surface sensitive (10-200 Å)

Elemental sensitivity (parts per 1000)

All elements (except H and He)

Quantitative

Chemical bonding information

Interpretation and theory straightforward

High information content

Disadvantages:

Large analysis area (several mm^2)

Expensive ($200,000-$500,000/instrument, $50-$500/sample)

High vacuum (10^{-8} to 10^{-11} torr)

Slow (1/2 to 8 hours/sample)

Charging and energy referencing can be a problem

Low resolution (~ 0.1-1.0 eV)

In our study, samples (1x1 cm^2) of polymer films were recorded using a Kratos XSAM-800 (Kratos, Manchester, England) XPS spectrometer employing an Mg $K_{\alpha_{1,2}}$ radiation and with a base pressure of about $5x10^{-8}$ torr and typical x-ray operating parameters of 12 kV and 17 mA. Data were stored and anlayzed on a DS 800 data system. The least-squares curve fitting program used for spectrum analysis could handle Gaussian, Lorentzian, and intermediate peaks. Due to the insulating nature of the samples the surface becomes positively charged when irradiated with x-rays. This charging effect leads to a decrease in

resolution and in a shift of the peaks toward higher binding energy (42). In order to improve the resolution, the spectra were recorded with the aid of an electron flood gun which provides low energy electrons for neutralization of the charging of the samples. The binding energy scale has been set by assigning a binding energy of 285.0 eV to the neutral carbon peak ($-CH_2-$).

3.2.9.3 SEM

Surface morphological data were obtained by scanning electron microscopy. Because of the insulating nature of the samples, all surfaces were coated with a Hummer V sputter coater (Technics, Alexandria, Virginia).

After preparation, each sample was examined using a JEOL JSM-35C SEM (JEOL, Boston, Massachusetts). Accelerating voltage was typically 5 kV. Varied magnifications were employed to best reveal surface morphological differences.

3.2.9.4 Contact angle

Information on the outermost few monolayers of solid surfaces is very difficult to obtain. One of the most sensitive methods known for obtaining true surface information is solid/liquid vapor at solid/liquid/liquid contact angle.

Captive air bubble contact angles were measured in order to estimate the extent of polarization of the oxidized LDPE. The contact angles were measured on a Rame-Hart contact angle goniometer (Mountain Lakes, New Jersey).

The LDPE films were held to the underside of a microscope glass slide with rubber bands and the slide was put across an acrylic immersion chamber (3x2x2-½) filled with doubly distilled water. The films were held submerged horizontally within the distilled water.

The LDPE films were equilibrated for approximately 6 hours before measurement to ensure complete surface hydration. A microsyringe containing air was used to form air bubbles underneath the hydrated surfaces. Angles on both sides of the bubble were measured assuring symetry. Typically 6 measurements were made.

3.2.9.5 Dye test

In this test, the oxidized and non oxidized films were put into contact with an aqueous solution (0.1%) of an alkaline dye, Rhodamine B, for several hours. The films were then removed and washed with water and ethanol. The homogeneity and intensity of color give qualitative information on the presence of carboxylic acid groups on the polymer surface (43).

4 RESULTS AND DISCUSSION

4.1 Chromic Acid Oxidation

The chemical modification of polymer surfaces has an extensive literature (44,45). The methods employed for a wide range of materials show often a high degree of commonality.

Considerable attention has been devoted to the use of chemical oxidation for surface modification of LDPE (46,42). Depending on the oxidizing agent used, and on conditions, the use of any chemical method results in high polarization of the surface.

Chromic acid oxidation is one of the most used methods. It has the following advantages: First, functionalization is restricted to the surface because of the hydrophobic nature of the substrate and the polar nature of the reagents and solvent which do not swell polyethylene. Therefore, the bulk properties of the polymer are not affected. Second, it is very convenient regarding the reaction conditions and relative ease of handling of chemicals. Third, it affords a relatively high density of carboxyl groups often higher than $2x10^{15}$ groups/cm^2 as determined by J. R. Rasmussen et al. using fluorescence spectroscopy (4,5). However, the method suffers from several disadvantages. First, any impurity may segregate at the surface, thus effecting reproductibility. Therefora, particular care must be taken during

sample preparation so that any surface contaminants must be removed. The second serious deficiency is that LDPE is structurally heterogeneous. LDPE is a mosaic of crystalline and amorphous regions having different structures and different relative reactivities (47). Chromic acid attacks preferentially the less ordered amorphous regions. However, it is expected that the crystalline regions show also some reactivity, particularly at the fold surface of the lamellar structure. Also, if any heterogeneity due to difference in reactivity, it might be minimized by the three-dimensional distribution of the carbonyl groups (Figure 10). Then this would lead to a totally oxidized surface. J. R. Rasmussen et al. estimated the functional group density as $2x10^{15}$ groups/cm^2 (4). Also, if one considers that the maximum packing density of oriented fatty acid in a monolayer is $5x10^{14}$ group/cm^2, it might be deduced a four times increase in area as a result of surface roughness of the film (Figure 10).

4.1.1 Mechanism

Usually the oxidation of hydrocarbons follows quite complex pathways (48). The mechanism of oxidation of alkanes has been reported by K. B. Wiberg and R. Eisenthal (49). In a simplified overall mechanism (Figure 11), the attack by Chromium VI proceeds through a Chromium IV ester intermediate to give an alcohol which is then further oxidized to give carboxyl, ketone, and aldehyde functionalities subsequently to a chain cleavage.

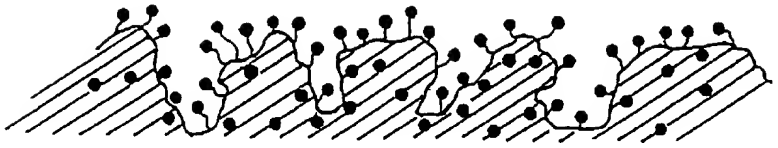


Figure 10. Schematic representation of the surface structure of the oxidized LDPE where (●) represent carbonyls.

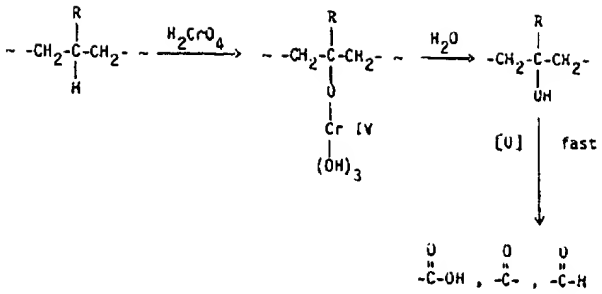


Figure 11. Chromic acid oxidation scheme for alkanes.
Source: Ref. 5

The differences in the rates and products of chromic acid attack on hydrocarbons is rationalized in the terms of relative oxidative resistance of primary, secondary, and tertiary -C-H bonds and the accessibility of chain segments to the oxidizing agents. The relative rates of ≡C-H, $-CH_2-$, and $-CH_3$ groups towards chromic acid oxidation in solution have been shown to be 7000:110:1, respectively (49). Therefore, the extent of oxidation and the homogeneity of the oxidized surface depend mainly on the number and the distribution of branches on the surfaces.

4.1.2 FT-IR/ATR

Figure 12 shows the FT-IR/ATR spectrum obtrained from the non oxidized LDPE film. All the features shown on the spectrum relate to the obsorption bands of the pure hydrocarbon. Table 1 shows the assignment of the major peaks.

The 1375 cm^{-1} band absorption is a good indication of the number of branches on the surface of LDPE. P. Blais et al. using the ATR technique have calculated the branch concentration on LDPE (50). The model compound 2-ethyl pentane was used to experimentally determine the extinction coefficient of $-CH_3$ absorption. The calculated branch concentration was 50±2 per 1000 $-CH_2-$ groups. The area of the $-CH_3$ absorption (1375 cm^{-1}) and the -CH band absorption (1460 cm^{-1}) obtained by P. Blais et al., compares very closely to the ratio obtained with our substrate. Therefore, we assume that the branch concentration of our LDPE is also about 50 per 1000 $-CH_2-$ groups.

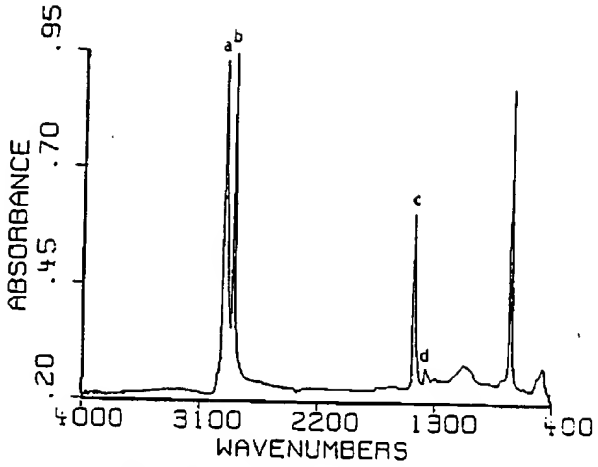


Figure 12. FT-IR/ATR spectrum for LDPE.

Table 1
Main FT-IR/ATR absorbtion bands for LDPE.

$\nu(cm^{-1})$	Peak		Assignment
2914	a	$-CH_2-$	asymmetric stretch
2844	b	$-CH_2-$	symmetric stretch
1460	c	-CH-	bend of $-CH_2-$
1375	d	$-CH_3-$	symmetric bend

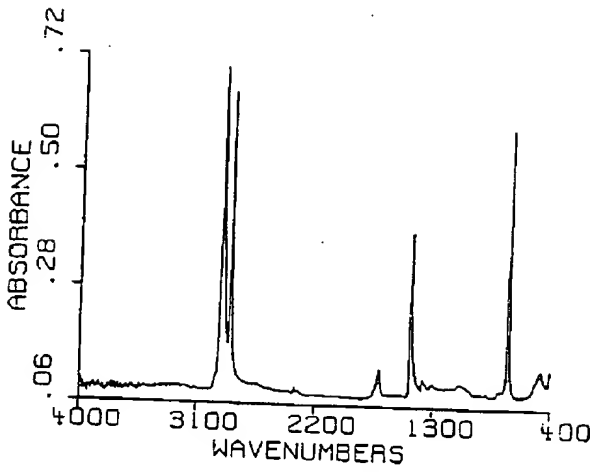


Figure 13. FT-IR/ATR spectrum for oxidized LDPE.

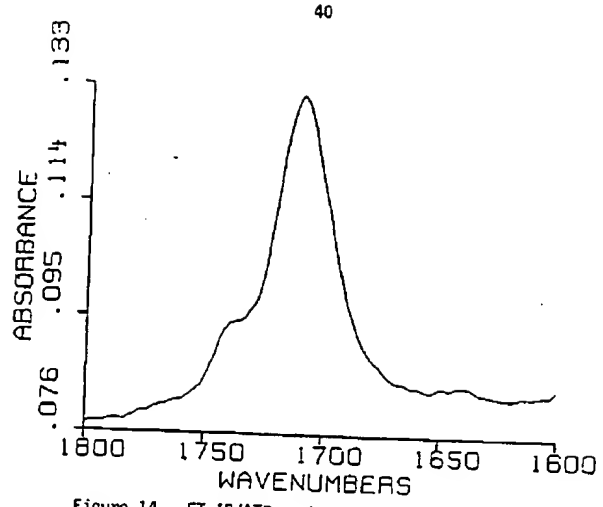


Figure 14. FT-IR/ATR carbonyl spectrum for oxidized LDPE.

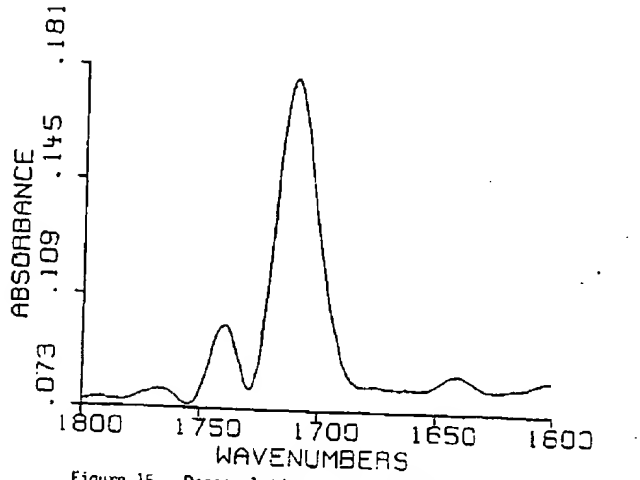


Figure 15. Deconvolution of the FT-IR/ATR carbonyl spectrum for oxidized LDPE.

Figure 13 shows a typical spectrum of oxidized LDPE. The main feature is the appearance of a new absorption band centered at 1712 cm^{-1}. This band corresponds to the carbonyl groups generated upon oxidation. Expansion of the carbonyl peak (Figure 14) and its computer deconvolution (Figure 15) shows two well separated peaks which are assigned to carboxylic groups at 1712 cm^{-1} and to aldehyde and ketone groups at 1725 cm^{-1}. These assignments were based on the derivatization carried out on surface aldehyde, ketone, and carboxyl groups done by J. R. Rasmussen (4). The ratio of the areas of the two carbonyl peaks gives:

$$\sim 80\% \ \overset{O}{\overset{\|}{-C}}-OH \text{ and } \sim 20\% \ \overset{O}{\overset{\|}{-C}}- + \overset{O}{\overset{\|}{-C}}-H$$

4.1.3 XPS

XPS analysis of the non oxidized LDPE (Figure 16) shows only a single major hydrocarbon peak centered at 285.0 ev. This spectrum shows a small oxygen signal (<1%). This small amount of surface oxidation could result from a variety of sources such as oxidation during film processing and storage, adsorbed water, etc. In any case, the small amount of oxygen contained on the non oxidized LDPE can be largely ignored in the deconvolution of the carbon 1s spectrum. The expansion of the C1s peak (Figure 17) shows an almost perfect symmetrical peak, charactertic of a $-CH_2-$ type of carbon.

Chromic acid treatment generates a significant amount of oxygen on the surface. Figure 18 shows the XPS data for oxidized LDPE at 72°C for 5 minutes. The amount of oxygen incorporated can vary as a function of

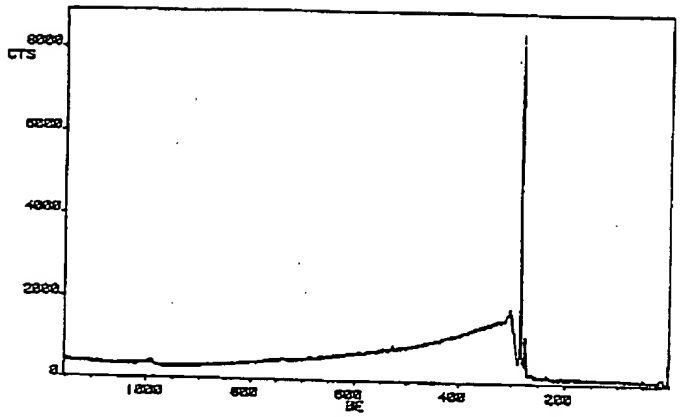


Figure 16. XPS spectrum for LDPE.

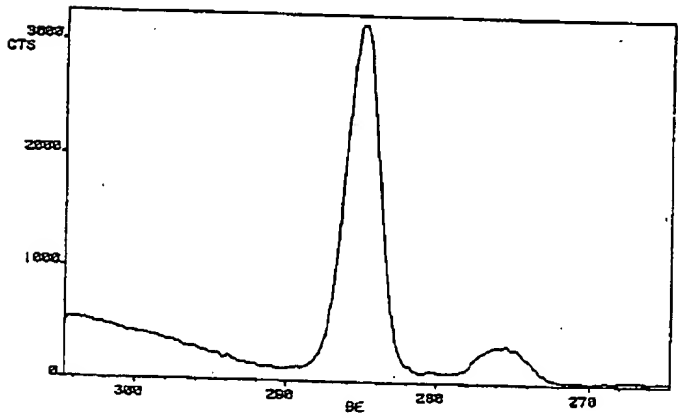


Figure 17. XPS C1S spectrum for LDPE.

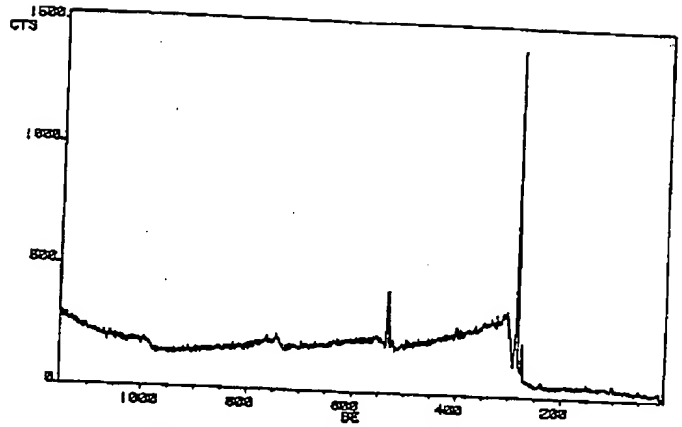


Figure 18. XPS spectrum for oxidized LDPE.

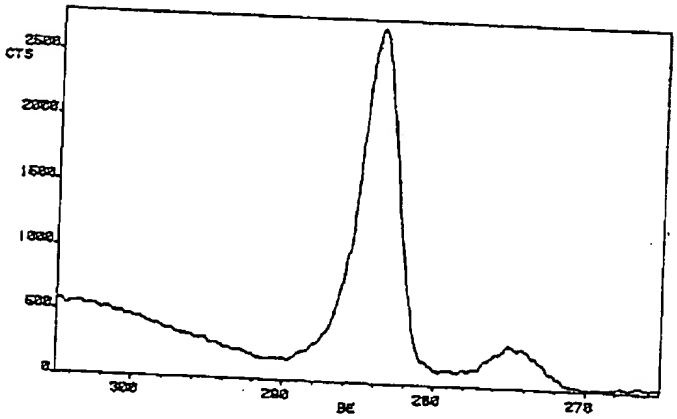
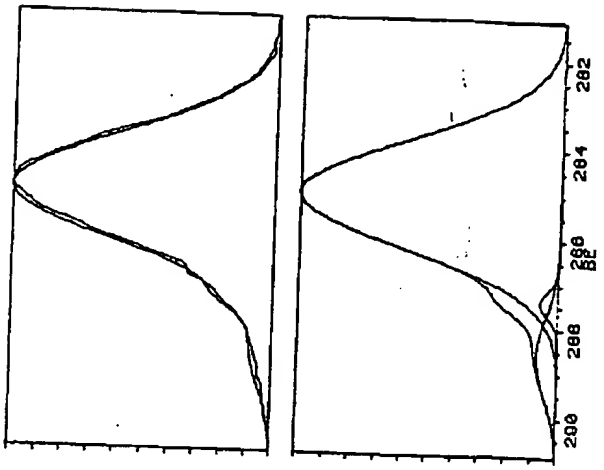


Figure 19. XPS C1S spectrum for oxidized LDPE.

Table 2

XPS Results--Atomic ratios of oxidized LDPE as a function of oxidation time.

Time (min.)	O_{1S}/C_{1S}
0	.020
.5	.115
1.0	.116
2.0	.110
3.0	.118
4.0	.119
5.0	.119
10.0	.129
15.0	.132
30.0	.137



Carbon-Type	Position (ev)	% Carbon Concentration
$-CH_2-$	285.	83.4
O -C-, -C-O-	287.5	1.7
O -C-OH	288.6	4.6

Figure 20. Deconvolution of XPS C1S for oxidized LDPE.

reaction time (Table 2). Small day-to-day variations in the amount of oxidation were also observed for identical reaction parameters. Generally, these differences were within ± 15% of a given value. Figure 19 shows the carbon 1s signal of the oxidized LDPE where an asymmetry can be noticed on the left of the peak. This extra structure is attributed to the oxygen functionality which shifts carbons to higher binding energies.

Based on the FT-IR/ATR data the asymmetry of the C1s peak can be due to a convolution of at least three distinct carbon or oxygen-bonded carbon species (51). Figure 20 shows a simple deconvolution of the high bending energy shoulder of the C1s peak. Three peaks emerge from the deconvolution which can be ascribed to $-CH_2-$ (pure hydrocarbon) at ≈ 285.0 ev, c = 0 (e.g. aldehyde, ketone) at ≈ 287.6 ev and $-\overset{\overset{O}{\|}}{C}-O-H$ at ≈ 288.7 ev. The relative areas of the different XPS C1S peaks (Figure 20) correlate quite well with the relative areas of the carbonyls determined by FT-IR/ATR (Figure 15). However, XPS is much less sensitive to the chemical shifts than the FT-IR/ATR.

4.1.4 Contact Angle

The wetting behavior and the extent of the oxidation is studied by contact angle measurements. Temperature was maintained constant at 72°C as determined by J. R. Rasmussen et al. (4) and time was varied from 0 to 30 minutes. Table 3 shows that the contact angle decreases sharply from 90° initially to 30° after only 1 minute of reaction time and then levels off after 5 minutes. This observation is consistent with the

Table 3

Contact angle measurements as a function of oxidation time.

Time (min.)	Contact Angle
0	90
.5	30
1.0	28
2.0	22
3.0	20
4.0	18
5.0	18
10.0	18
15.0	18
30.0	18

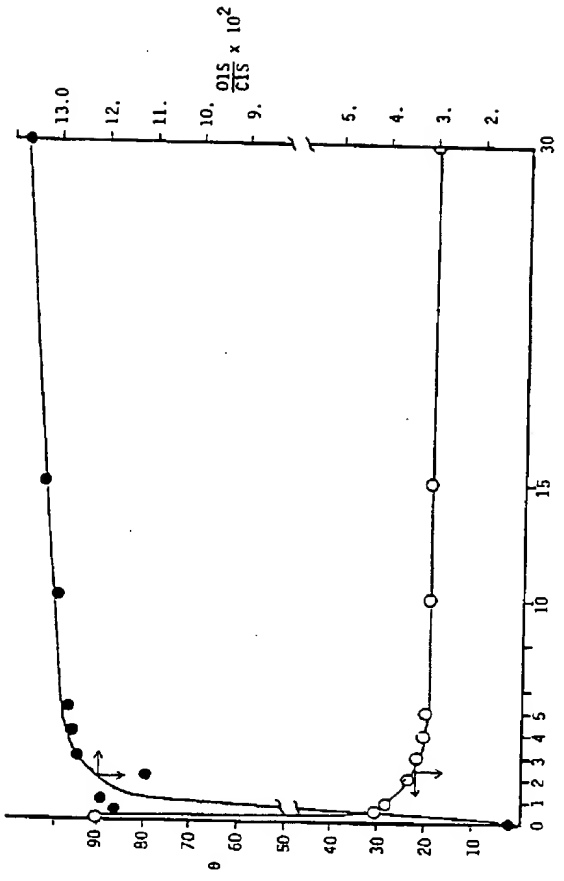


Figure 21. Contact angle (O) and XPS O1S/C1S ratios (●) as a function of oxidation time for LDPE.

sampling depth of the technique which is sensitive only to the outermost one or two atomic layers (2). Therefore, the oxidation of the surface reaches its maximum at 5 minutes, which after continues deeper into the bulk.

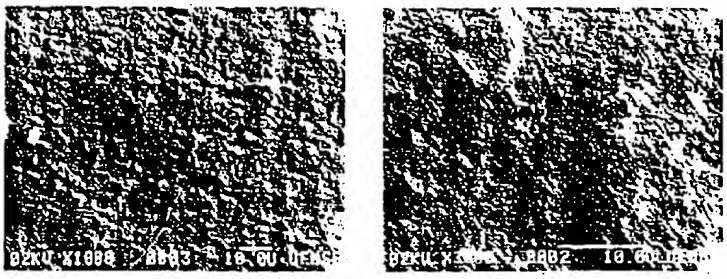
The observed changes in contact angle clearly suggests that the surface is highly hydrophilic and made polar by the chromic acid oxidation.

Figure 21 represents a combination of the XPS and contact angle measurements. The O1s/C1s intensity ratio is plotted as a function of oxidation time, with the contact angle of the air bubble. The initially rapid increase in the O1s/C1s ratio starting to level off after about 5 minutes of treatment, correlates well with corresponding decreases in contact angles. While LDPE continues to oxidize in depth with exposure time, the XPS data suggest that an equilibrium layer of oxidized polymer is being reached after 5 minutes of oxidation time. The equilibrium is established by a balance between the newly formed carbonyl groups and the ones leached into solution.

The above ESCA and contact angle data correlate very well with the results obtained by J. R. Rasmussen et al. (4,5) using fluorescence spectroscopy.

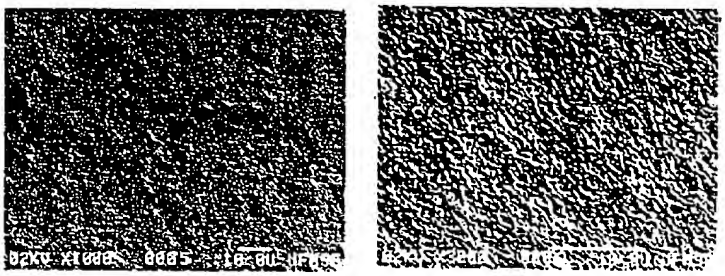
4.1.5 SEM

In order to determine morphological differences bertween the non oxidized and oxidized LDPE films, samples were examined by scanning electron microscopy at two different magnifications. Figures 22 (a & b)



(a) (b)

Figure 22. Scanning electron micrographs of LDPE.
(a) 1000 X (b) 3000 X



(a) (b)

Figure 23. Scanning electron micrographs of oxidized LDPE.
(a) 1000 X (b) 3000 X

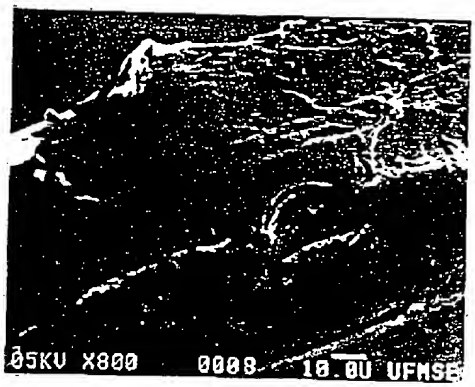


Figure 24. Scanning electron micrograph of a cross section for oxidized LDPE.

show the surface of non oxidized LDPE film. The surface appears relatively smooth and regular. Figures 23 (a & b) show a rough and pitted oxidized LDPE surface. This is consistent with the loss from the amorphous areas of large fragments of tie molecules, or branches, which previously were covering any surface imperfections. Figure 24, representing a cross section of the LDPE film sitting parallel to the electron beam, shows that the bulk is not affected by the oxidation.

4.1.6 Dye Test

Oxidized and non oxidized LDPE films are put into an aqueous solution of Rhodamine B for a few hours. The films are then removed and washed with water and ethanol. The test shows a rapid and intense coloration of the oxidized LDPE film while the non oxidized film shows no coloration. The test confirms the presence of carboxylic groups on the surface (43).

4.2 Amido-Amine Modified LDPE Surface

The carobxylic acid moieties can be converted in high yields into a variety of useful derivatives. One of the most successful routes for derivatization of the carboxylic groups is based on the use of dicyclohexylcarbodiimide (DCC) as a dehydrating agent. The acid group are reacted with an amine and the amide linkage is usually formed in good yields.

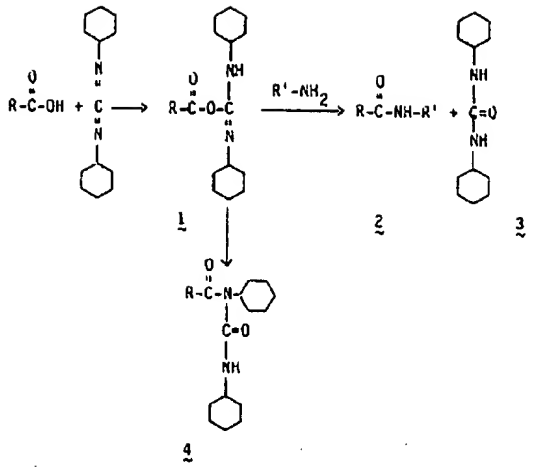
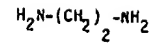


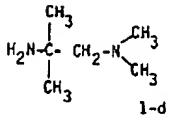
Figure 25. Mechanism scheme of coupling of a carboxylic group with an amine.

A



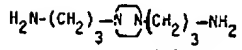
Etnylene diamine

B



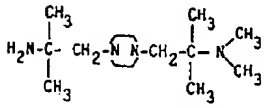
1-dimethylamino-2-amino-2-methylpropane

C



Bis(propylamine) piperazine

D



1,4-Bis(2-amino-2-methylpropyl) piperazine

Figure 26. Different polyamines grafted onto oxidized LDPE.

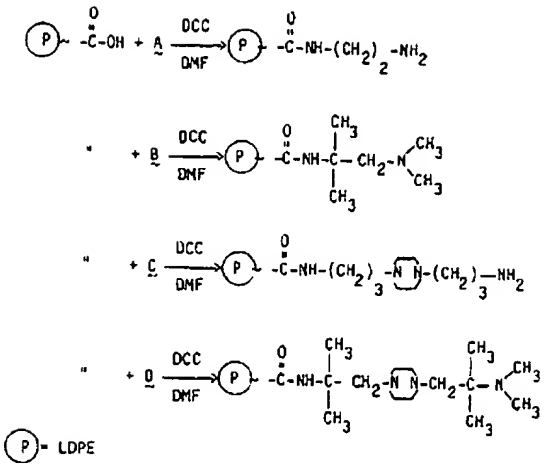
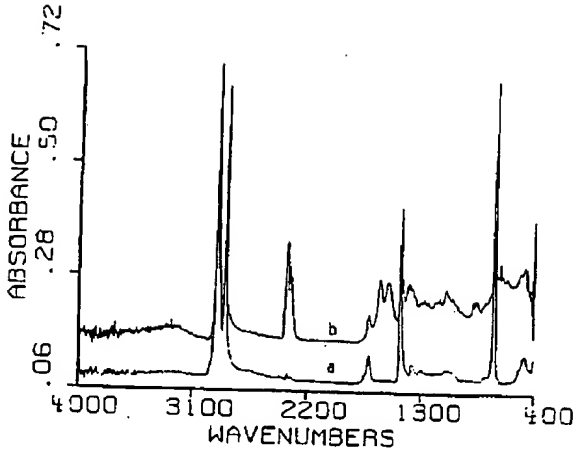


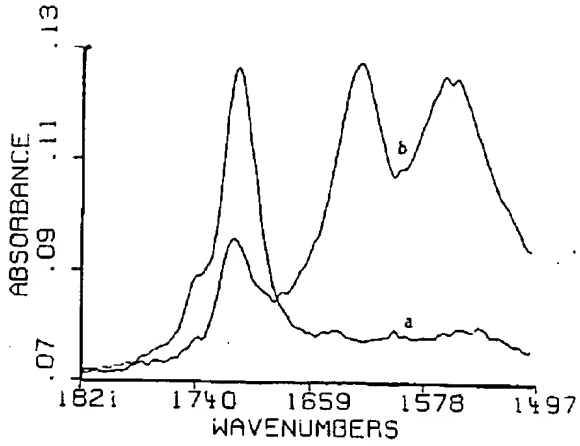
Figure 27. Different amido-amine modified LDPE.

4.2.1 Mechanism

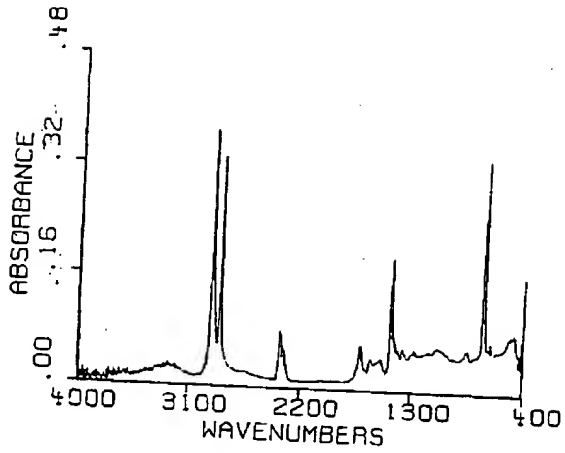
The reaction mechanism (Figure 25) was thoroughly investigated by DeTar et al. (52). The first step involves addition of the acid to the reagent to form a reactive intermediate, the O-acylisourea 1. A frequently found by-product is the N-acylurea, structure 4 in Figure 25, which can be produced by an O → N acyl migration (32). Because of its stability, structure 4 is unreactive towards amines and do not form amide bonds. However, formation of the N-acylurea could be easily overcome by operating at low temperature (0°C) in order to stabilize the reactive ester intermediate 1. Also, the choice of solvent has an effect on the reaction path. An aprotic solvent, for instance, favors the dimeric structure of the carboxylic acid groups, while the monomeric form is favored by a protic solvent. In the monomeric form, the carboxylic acid is highly hydrogen-bonded with the solvent, therefore, the nucleophilicity of the acid is highly decreased (22). The polarity of the solvent has also been pointed out to play a major role in the kinetics and the efficiency of the coupling reaction (53).

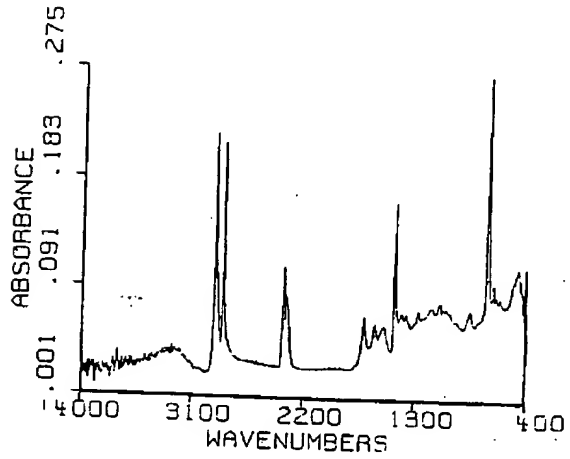
In our investigation, we attempted the derivatization of the carboxylic groups generated on the LDPE surface. We used DMF as an aprotic and polar solvent. A series of four amines (Figure 26) with different structures and different steric hindrance were used. The expected result is the covalent attachment of the amine through an amide linkage and maintaining a terminal unreacted amine group (Figure 27) for further reactions.


.72
.50
.28
.06
ABSORBANCE
b
a
4000
3100
2200
1300
400
WAVENUMBERS


.13
.11
.09
.07
ABSORBANCE
b
a
1821
1740
1659
1578
1497
WAVENUMBERS

60


.48
.32
.16
.00
ABSORBANCE
4000
3100
2200
1300
400
WAVENUMBERS


.275
.183
.091
-.001
ABSORBANCE
4000
3100
2200
1300
400
WAVENUMBERS

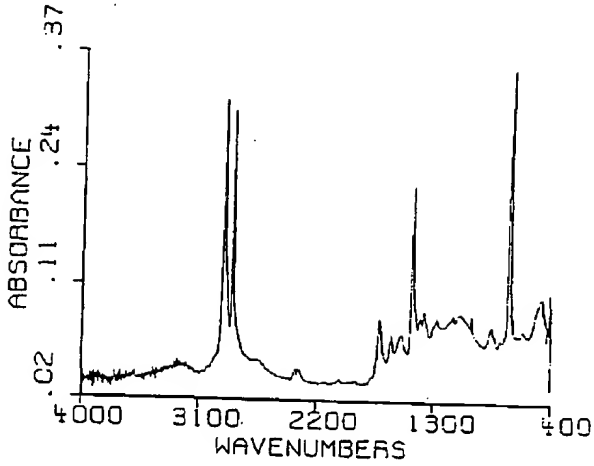
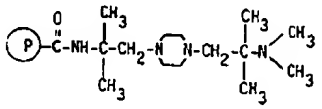


Figure 32. FT-IR/ATR spectrum for



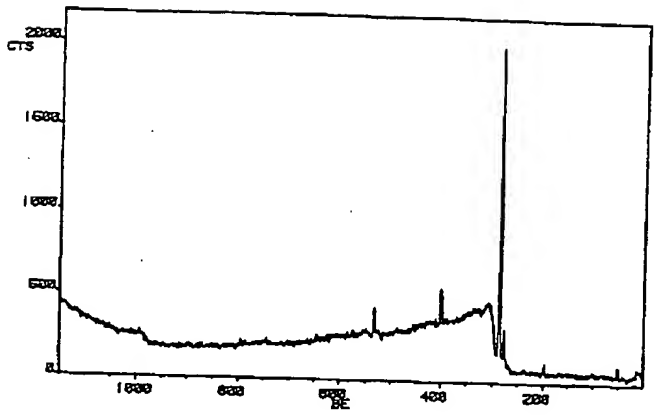


Figure 33. XPS spectrum for Ⓟ–C(=O)–NH–$(CH_2)_2$–NH_2

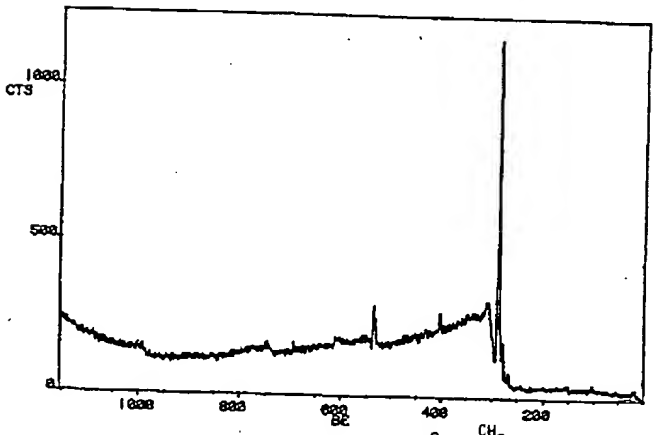


Figure 34. XPS spectrum for Ⓟ–C(=O)–NH–C(CH_3)(CH_3)–CH_2–N(CH_3)(CH_3)

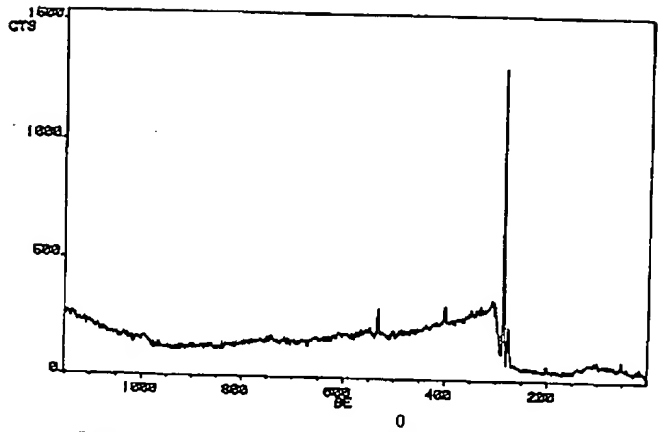


Figure 35. XPS spectrum for Ⓟ–C(=O)–NH–$(CH_2)_3$–N⬡N–$(CH_2)_3$–NH_2

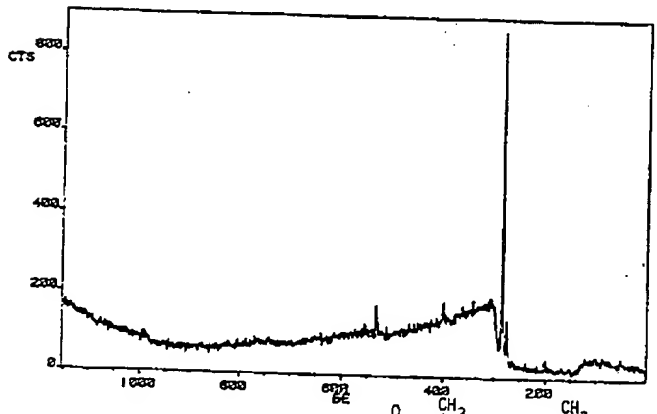


Figure 36. XPS spectrum for Ⓟ–C(=O)–NH–C(CH_3)(CH_3)–CH_2–N⬡N–CH_2–C(CH_3)(CH_3)–N(CH_3)(CH_3)

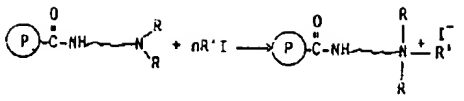
4.2.2 FT-IR/ATR

Absorbance spectra of all the amido-amine modified LDPE surfaces are shown in Figures 28, 29, 30, 31, and 32. In particular, Figure 28 compares the oxidized LDPE surface (spectrum (a)) and the amido-ethylene amine modified LDPE surface (spectrum (b)). Expansion fo the carbonyl regions is shown in Figure 29. Clearly, we notice a decrease in intensity of the 1712 cm^{-1} band (residuel $-\overset{O}{\overset{\|}{C}}-OH$ and unreacted $-\overset{O}{\overset{\|}{C}}-$ and $-\overset{O}{\overset{\|}{C}}-H$) in spectrum (a), while an amide type structure with characteristic bands at 1625 cm^{-1} (amide carbonyl) and 1558 cm^{-1} (amide -NH) in spectrum (b).

Comparison of bond positions in Figure 28, 30, 31, and 32 indicate that the amide covalent bond is formed in all cases. Examination of the absorbances of the carbonyl peak at 1712 cm^{-1} (residual $-\overset{O}{\overset{\|}{C}}-OH$, non reacted $-\overset{O}{\overset{\|}{C}}-$ and $-\overset{O}{\overset{\|}{C}}-H$) and the amide carbonyl at 1625 cm^{-1} provide some quantitative insight into the extent of the reactions in all four cases. Clearly, the amide carbonyl formed with ethylendiamine (Figure 29b) is more intense than with the three other polyamines. Although secondary amines are stronger nucleophiles than primary amines, steric hindrance seems to be a more important factor in a heterogeneous system.

4.2.3 XPS

The XPS survey spectra (Figures 33-36) show that the nitrogen, with a binding energy of 401 ev, is incorporated in all cases. The oxygen content which is about 11% for the oxidized LDPE decreases to 6-9% for the amido-amine modified surfaces. This is consistent with the loss of



R = H, $-CH_3$

R' = $-CH_3$, $-C_2H_5$

Figure 37. Potential alkylation path for an amido-amine surface.

the hydroxyl of the carboxylic group for each coupling reaction. According to the quantitative XPS analysis in Table 4, the ethylenediamine gave the better yield if we look at the oxygen and nitrogen contents. However, it will be difficult to draw quantitatively any comparison between the three other polyamines for the experimental error could be nigher than 20% according to our experience.

4.3 Quaternization of the LDPE Surface

4.3.1 Alkylation of the Amido-Amine Modified LDPE

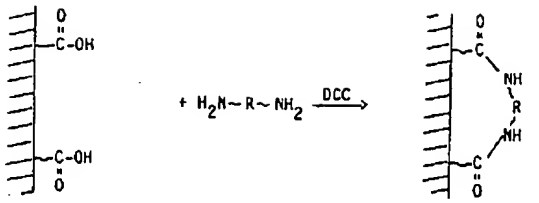
Subsequent to the grafting of the amido-amine groups on the LDPE surface we attempted to quaternize the LDPE via the terminal amine group covalently attached to the surface. The reaction which might take place is shown in Figure 37.

In solution, the quaternization of a dialkyl amine, for instance, proceeds quite readily with relatively high conversions also under mild conditions (33,34). The product is easily characterized by several methods. For instance XPS will detect easily the halide counterion. Also, chemical shifts due to the cationic character of the nitrogen can be easily seen with ^{1}HNMR.

Quaternization of amines attached to a solid substrate has not been reported in the literature as far as we could determine. However, several other kinds of organic synthesis in solid state phase are reported (47,54). All these studies concluded that there is no systematic correlation between solution chemistry and solid state chemistry.

In our attempts to quaternize (at room temperature) the amines attached to solid polyethylene according to the principles of solution chemistry, we obtained no evidence for such reaction. The XPS data show no iodide, as counterion for the ammonium salt, if any, on the surface. Also, a quaternary ammonium group should provide high hydrophilicity with a contact angle near zero. The wetting behavior of our samples did not change. The presence of the organic solid substrate, we feel, is somehow inhibiting this reaction.

It is reported (48,54) that the rate of chemical reaction at an interface might increase as in heterogeneous catalytic processes, enzymatic reactions, etc., or might decrease due to reduction of the relative accessibility and orientation of reactants. The motion of the reactants is also drastically decreased by the reduction of dimensionality of space. Consequently, the forces of solvation are also attenuated. Another reason for failure in a heterogeneous synthesis could come from the surface physics which might perturb reactivity as well: polymers tend to concentrate non polar functionality at their surfaces to minimize surface energy (35). Also, in the grafting of the amido-amine groups, we cannot rule out the possibility that both the terminal amine groups can react to form a surface with no nucleophilic sites. This reaction is possible in at least two cases: with ethylenediamine and bis (aminopropyl) piperazine as shown below:



More severe reaction conditions such as rise in temperature up to 60°C and longer reaction time up to 7 days, though could have provided more mobility, better solvation, then acceleration of the alkylation of the amine group had no effect, but development of side reactions of the alkylating agent. Also, if any ammonium groups are formed, degradation via Hoffman's mechanism is a possibility. As a consequence of all these difficulties we had to resort to another alternative such as a grafting of a vinyl monomer bearing a trialkyl ammonium group.

4.3.2 Plasma-Induced Graft Polymerization of Vinyl Monomer Salt onto LDPE

The synthesis of quaternary ammonium salts of dimethylaminoethyl methacrylate (DMAEMA) and their polymerization have been extensively investigated and described in the patent literature (55,23). Most of the monomeric salts are of a highly hydrophilic character and find a wide range of applications such as water treatment, soil conditioning, antistatic treatment of textiles, etc. (23).

On the other hand, very little is known about the graft polymerization of quaternary ammonium salts of DMAEMA onto solid surfaces.

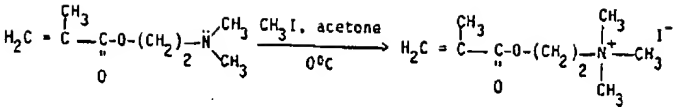


Figure 38. Synthetic scheme of alkylation of DMAEMA.

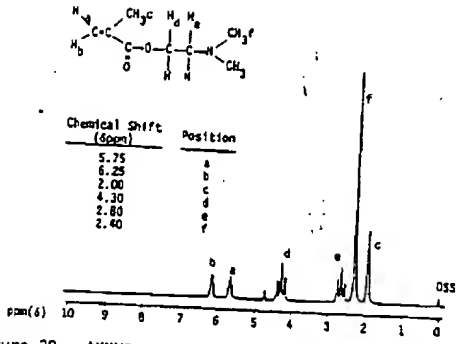


Figure 39. ^{1}HNMR 60 MHz spectrum for DMAEMA in D_2O at 25°C.

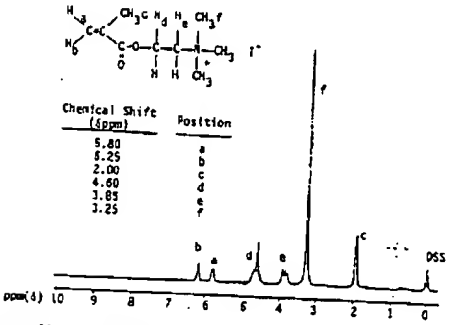


Figure 40. ^{1}HNMR 60 MHz spectrum for Q-DMAEMA in D_2O at 25°C.

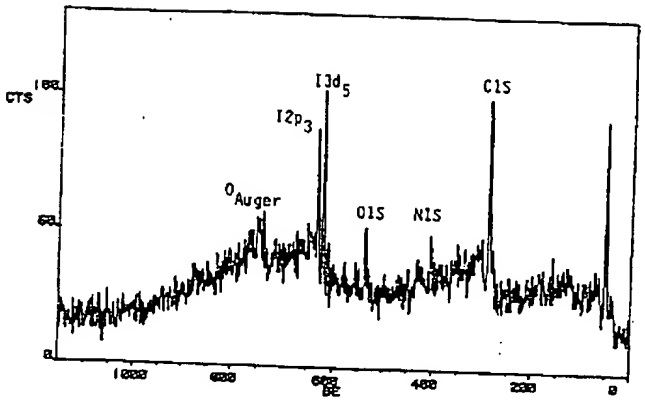


Figure 41. XPS spectrum for Q-DMAEMA.

Table 5
XPS Results--Atomic concentrations and ratios for Q-DMAEMA.

% Atomic Concentration				Atomic Ratios		
C	O	N	I	O/C	N/C	I/C
74.53	14.06	5.98	5.43 (a)	.189	.080	.073
69.20	15.4	7.7	7.7 (b)			

(a) = observed
(b) = calculated

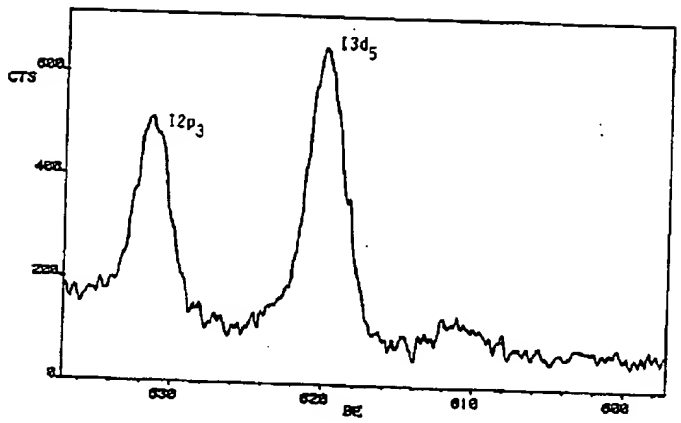


Figure 42. XPS spectrum of iodide for Q-DMAEMA.

4.3.2.1 Quaternized monomer synthesis and characterization

The alkylation of DMAEMA with methyl iodide (Figure 38) was carried out in acetone and at 0°C because the reaction is highly exothermic. The reaction was carried out in less than 2 minutes and in high yields. The monomer salt was characerized by ^{1}HNMR (60 MHz) in D_2O and by XPS.

Figures 39 and 40 show the ^{1}HNMR spectra of DMAEMA and Q-DMAEMA. A comparison of the two spectra shows that the methylene and methyl protons near the quaternary ammonium group are shifted downfield because of the cationic character of the nitrogen.

The XPS spectrum of Q-DMAEMA in the powder form is shown in Figure 41. It can be seen that the iodide peaks emerge at about 620 and 631 electron volts for the $I3d_5$ and $I2p_3$ electron orbitals, respectively. Notice that the high noise level in that spectrum is mainly due to the rough surface of the powder form of the analyte. Figure 42 shows an expansion of the iodide peaks. The quantitative XPS analysis (Table 5) indicates that about the same content of nitrogen (5.98%) and iodide (5.43%) incorporated to the surface. Taking into consideration the experimental error margin, this result is in good agreement with the theoretical stoichiometry which is a 1:1 ratio between iodide and nitrogen.

4.3.2.2 Plasma-induced graft polymerization

The plasma-induced graft polymerization technique involves a separation of the cold plasma treatment and grafting processes. The solid LDPE substrate is first plasma treated alone (under vacuum) and

Table 6

Results of plasma-induced grafting of vinyl monomers onto LDPE.

Monomer	Monomer Concentration in Water	Grafting*	XPS Atomic Ratios N/C	I/C
DMAEMA	100%	No	-	-
Q-DMAEMA	50 wt.%	No	-	-
Q-DMAEMA	10 wt.%	No	-	-
AM	20 wt.%	Yes	.071	-
[AM]/[Q-DMAEMA]=2		Yes	.018	.01
[AM]/[Q-DMAEMA]=10		Yes	.028	.01
[AM]/[Q-DMAEMA]=20		Yes	.020	.02

* Reaction Conditions

Plasma: Power = 25 watts
Pressure = 10^{-2} torr
Time = 90 seconds
Gas = Argon

Post-Polymerization: under vacuum, room temperature, 12 hours

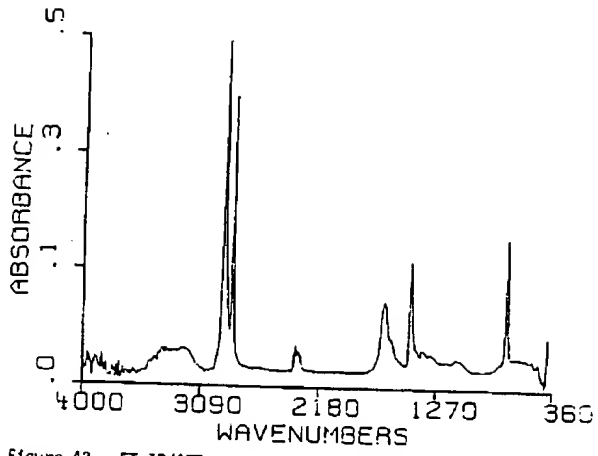


Figure 43. FT-IR/ATR spectrum for plasma-induced graft polymerization of AM onto LDPE.

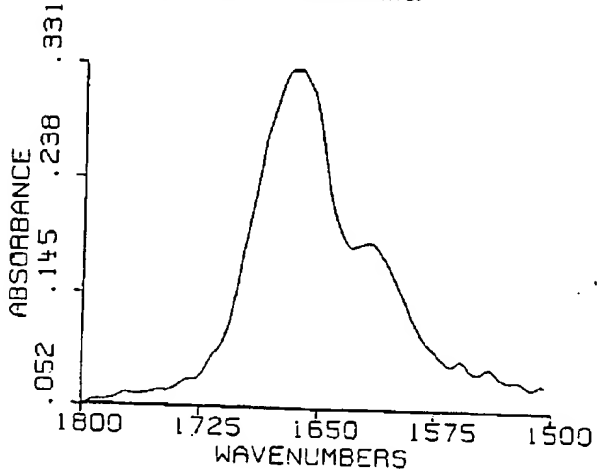


Figure 44. FT-IR/ATR carbonyl spectrum for plasma-induced graft polymerization of AM onto LDPE.

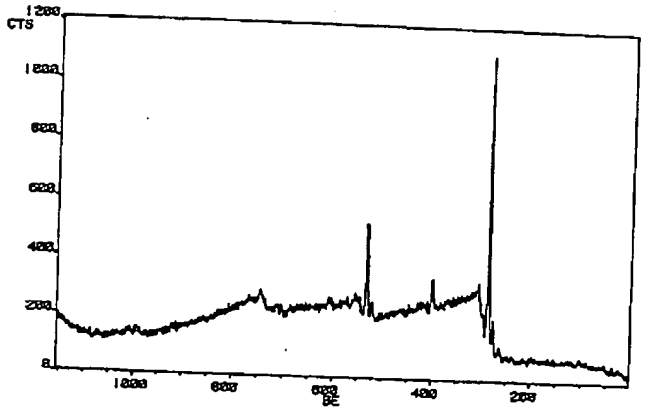


Figure 45. XPS spectrum for plasma-induced graft polymerization of acrylamide onto LDPE.

Table 7

XPS Results--Atomic concentrations and ratios for plasma-induced polymerization of AM onto LDPE.

% Atomic Concentration			Atomic Ratios	
C	O	N	O/C	N/C
80.01	14.3	5.7	.179	.071

then contacted with monomer. Graft polymerization is initiated by small concentrations of long-lived active species formed by the radiolytic bond-breakage of polyethylene and which remain trapped in the solid polymer (27,56).

The monomers used were: dimethyl amino-ethyl-methacrylate (DMAEMA), trimethyl quaternary ammonium salt of DMAEMA (Q-DMAEMA), acrylamide (AM), and also combinations such as AM/Q-DMAEMA.

Table 6 shows the preliminary grafting results and reaction conditions for the various monomers used. It could be seen that monomers such as DMAEMA and its ammonium salt (Q-DMAEMA) could not be grafted, although they are easily homopolymerized in a homogeneous system (23). However, AM was effectively graft polymerized onto LDPE. The FT-IR/ATR data (Figure 43,44) clearly show the appearance of an intense primary amide absorption band at about 1660 cm^{-1} and 1590 cm^{-1}. Also, notice the $-NH_2$ absorption band between 3100 and 3500 cm^{-1}. Repeated washing of the graft with water decreased only slightly (<10%) the intensity of the amide peak.

The XPS spectrum (Figure 45) of the AM-grafted LDPE shows three major peaks: carbon 1s at 285 ev, oxygen 1S at 531 ev, and nitrogen 1s at 401ev. From a theoretical standpoint, we expected a 1:1 ratio between the nitrogen and the oxygen. However, the experimental quantitative XPS data (Table 8) shows a higher oxygen than nitrogen content on the surface ($\frac{\%O}{\%N} = 2.5$) . This discrepancy might be due to water strongly adsorbed within the highly hydrophilic coating of polyacrylamide.

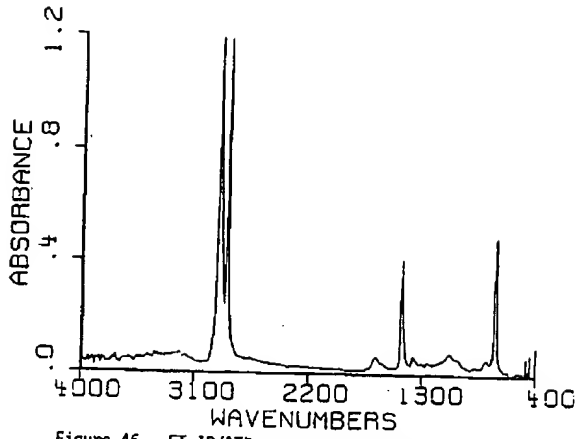


Figure 46. FT-IR/ATR spectrum for plasma-induced graft copolymerization of AM/Q-DMAEMA onto LDPE.

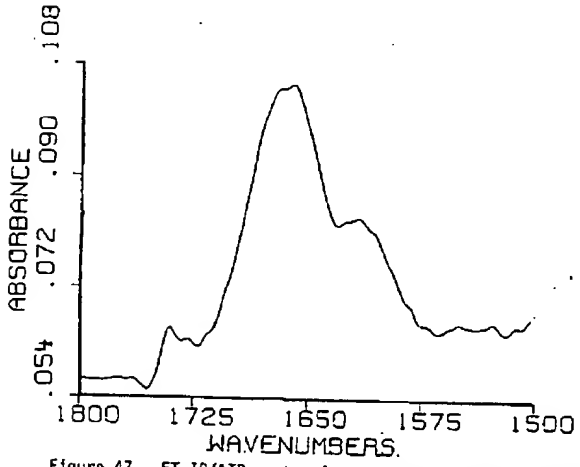


Figure 47. FT-IR/ATR carbonyl spectrum for plasma-induced graft copolymerization of AM/Q-DMAEMA onto LDPE.

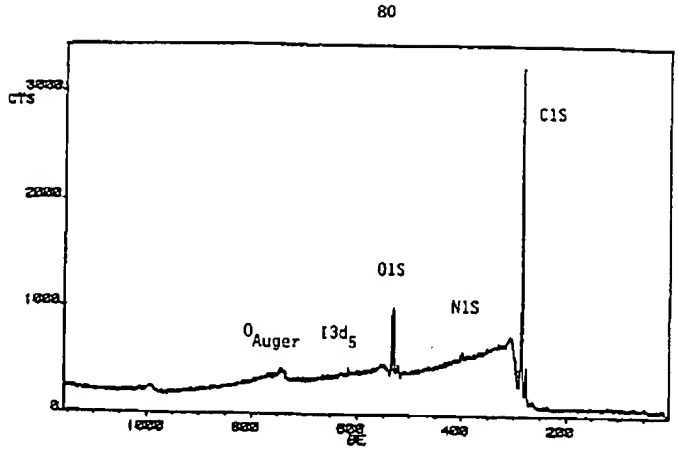


Figure 48. XPS spectrum for plasma-induced graft copolymerization of AM/Q-DMAEMA onto LDPE.

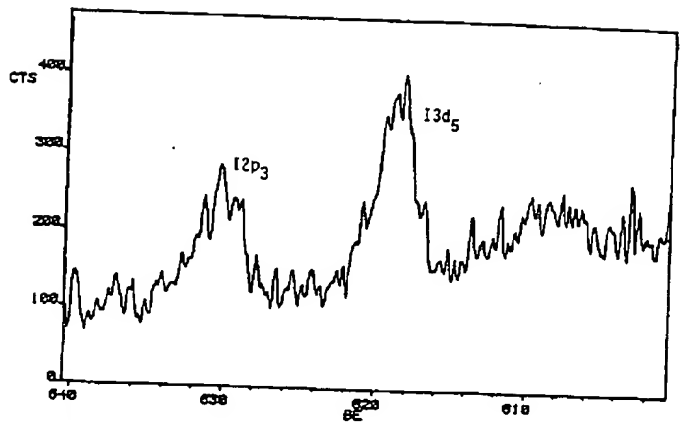


Figure 49. XPS iodide spectrum for plasma-induced graft copolymerization of AM/Q-DMAEMA onto LDPE.

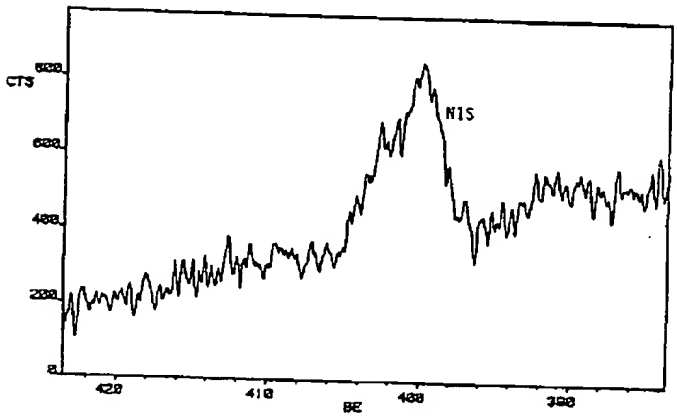


Figure 50. XPS nitrogen spectrum for plasma-induced graft copolymerization of AM/Q-DMAEMA onto LDPE.

On the other hand, graft copolymerization of Q-DMAEMA and AM also gave positive results. The FT-IR/ATR analysis (Figure 46) shows the appearance of a broad carbonyl peak. Expansion of the 1800-1500 cm^{-1} region (Figure 47) clearly shows three absorption bonds: at 1739 cm^{-1} a weak peak for the $-\overset{O}{\overset{\|}{C}}-O-$ of Q-DMAEMA, and at 1662 cm^{-1} and 1590 cm^{-1} for the $-\overset{O}{\overset{\|}{C}}-NH_2$ of AM. The intensity of these peaks correlates fairly well with the molar ratio used ([AM]/[Q-DMAEMA] = 20).

Figure 48 shows the XPS spectrum of the AM/Q-DMAEMA grafted LDPE. The iodine and nitrogen peaks at 619 and 401 ev, respectively, can be barely seen due to the low concentration of these elements on the surface. The expansion of the iodine and nitrogen peaks is shown in Figures 49 and 50.

Both the FT-IR/ATR and XPS data provide evidence for the graft copolymerization of AM and Q-DMAEMA. However, both methods show a lower grafting yield than with the acrylamide alone. The inhibition of the growing chain could be attributed to the Q-DMAEMA monomer because somewhat better grafting is obtained with only AM or where AM is used in excess during copolymerization (Table 6). Several reasons could be considered for the low graft copolymerization of Q-DMAEMA. First, the monomer is sterically hindered by the bulky trialkyl ammonium group, which then decreases the rate of propagation of the polymer chain (53). Second, the surfactant nature of the monomer might lead to micelle formation. It has been reported that the kinetic behavior of polymerization of certain vinyl pyridinium salts is drastically decreased by monomer aggregation (57). Third, chain transfer reaction

from the growing methacrylate to monomer or solvent is also possible (53). Since no initiator is present, no reinitation is possible. Fourth, the reaction conditions (time, temperature, inert atmosphere) are yet to be optimized. Fifth, there is still a controversy regarding the nature of the active species as already mentioned in Section 3.2.3.3.

4.4 Graft Polymerization of Acrylamide onto LDPE by Ce^{4+} Initiation

Redox initiation is often an efficient method of initating graft polymerization. Hydroxyl-containing polymers such as poly (vinyl alcohol), cellulose, and starch undergo redox reaction with ceric ion to form polymer radicals. In our study, we generated hydroxyl groups on the LDPE surface and were able to graft polymerize acrylamide onto it.

4.4.1 Diborane Reduction of Oxidized LDPE

In order to generate hydroxyl groups on the LDPE, we used the diborane/THF complex to reduce the carbonyls present on the oxidized LDPE to alcohols (Figure 51, Step I). Diborane is known to selectively reduce carboxylic groups, however, extensive reduction also converts aldehydes and ketones to alcohols (35).

The effect of the diborane reduction is well illustrated by the FT-IR/ATR analysis. Figures 52 and 53 refer to the oxidized and reduced films, respectively. In Figure 53, the carbonyl peak disappeared almost completely. The FT-IR/ATR analysis does not show yet the absorption peak of the hydroxyls, probably because their absolute number on the

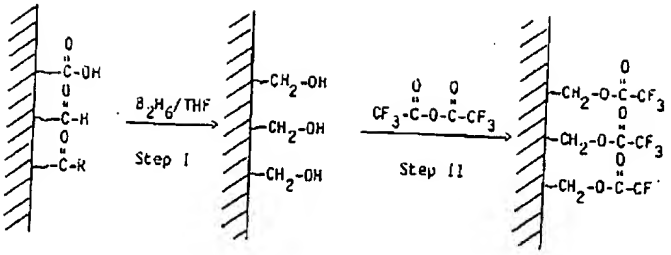


Figure 51. Scheme of diborane reduction of carbonyls and derivatization.

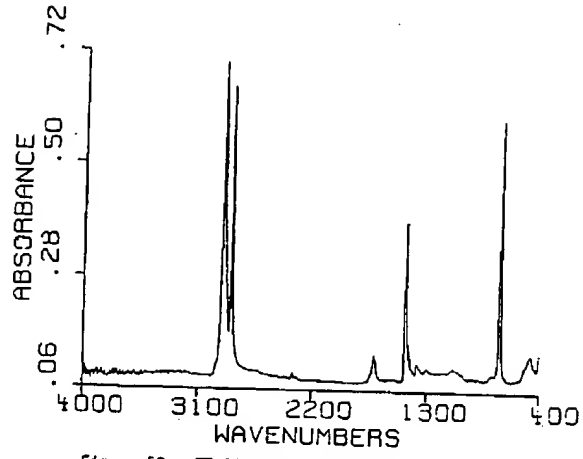


Figure 52. FT-IR/ATR spectrum for oxidized LDPE.

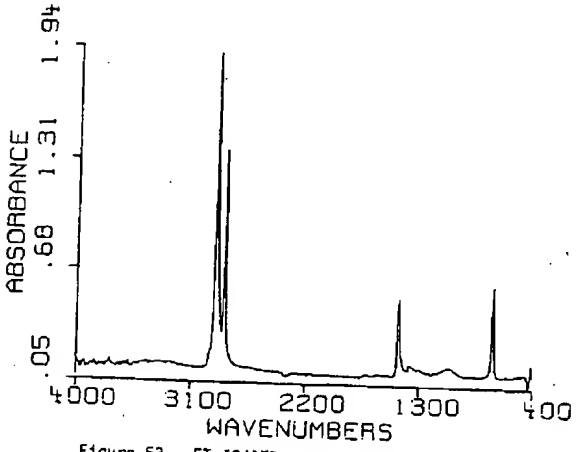


Figure 53. FT-IR/ATR spectrum of oxidized LDPE after diborane reduction.

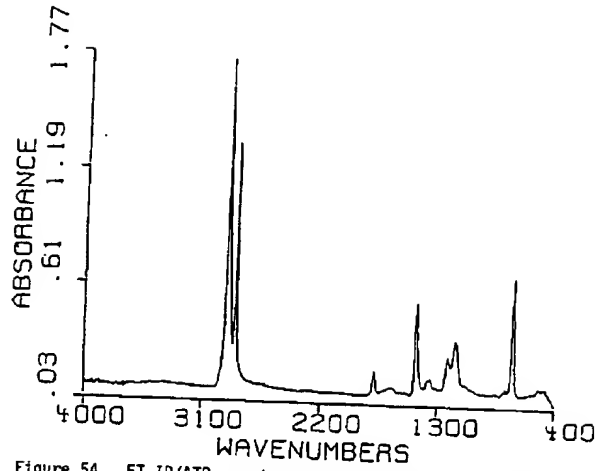


Figure 54. FT-IR/ATR spectrum for the fluoroester modified LDPE.

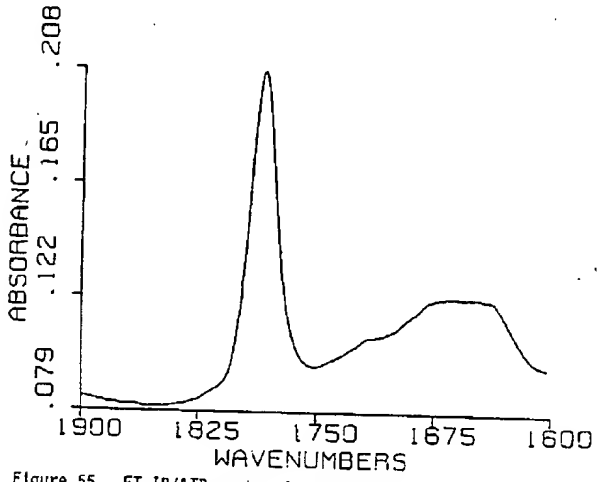


Figure 55. FT-IR/ATR carbonyl spectrum for the fluoroester modified LDPE.

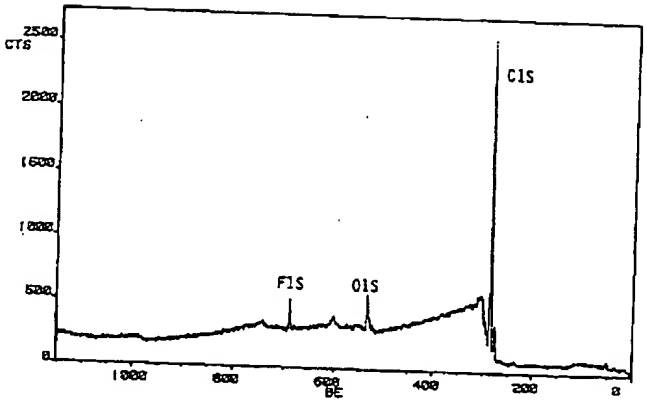


Figure 56. XPS spectrum for the fluoroester modified LDPE.

Table 8

XPS Results--Atomic concentration and ratios for the fluoroester modified LDPE.

% Atomic Concentration			Atomic Ratios	
C	O	F	O/C	F/C
88.2	7.1	4.7	.081	.053

surface is not high enough to be detected by ATR technique (4). However, we resorted to a derivatization technique to bring evidence of the alcohol-like LDPE surface.

4.4.2 Derivatization of Surface Alcohols

In order to obtain evidence for the presence of hydroxyl group on the LDPE surface we used a derivatization technique. Trifluoroacetic anhydride was reacted with the hydroxyls on the surface to yield ester linkages (Figure 51, Step II). This reaction was easily carried out and the fluoroester groups show very well at 1784 cm^{-1} in the FT-IR/ATR spectra (Figures 54 and 55). Figure 55 shows the expansion of the carbonyl peak where a shift to higher wavenumber (1784 cm^{-1}) is seen. This shift and the high intensity of the ester group is due to the higher dipole moment change induced by the highly electronegative fluorine atoms.

The XPS analysis (Figure 56 and Table 8) confirms the formation of the fluorinated ester. The florine 1S and oxygen 1S peaks can be seen at 689 ev and 531 ev, respectively.

Both the FT-IR/ATR and XPS data give clear evidence that the reduction of the surface carbonyls ($\overset{O}{\overset{\|}{-C-OH}}$, $\overset{O}{\overset{\|}{-C-}}$, $\overset{O}{\overset{\|}{-C-H}}$) led to an alcohol-like surface.

4.4.3 Graft of Polyacrylamide onto LDPE

Graft copolymerization of vinyl monomers onto hydroxyl-bearing substrates by Ce^{4+} induced initiation is an effective synthetic method

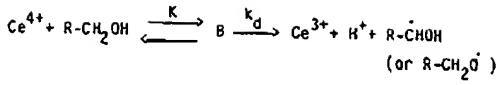


Figure 57. Interaction scheme between Ce^{4+} and an alcohol.

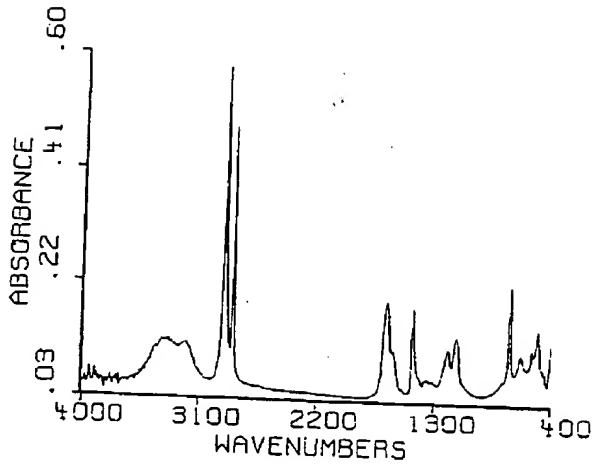


Figure 58. FT-IR/ATR spectrum for Ce^{4+}-induced graft polymerization of AM onto LDPE.

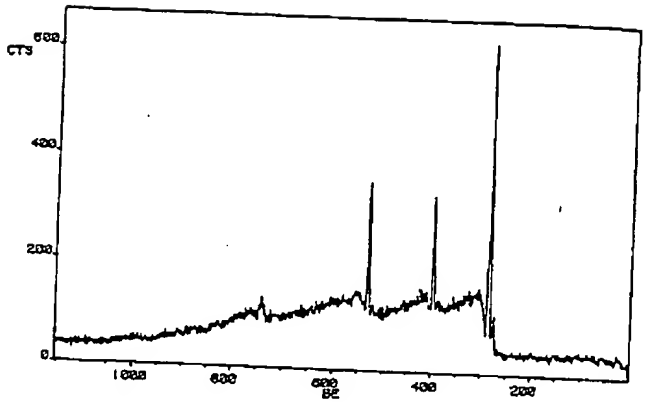


Figure 59. XPS spectrum for Ce^{4+}-induced graft polymerization of AM onto LDPE.

Table 9

XPS Results--Atomic concentrations and ratios for Ce^{4+}-induced graft polymerization of AM onto LDPE.

% Atomic Concentration			Atomic Ratios	
C	O	N	O/C	N/C
69.8	14.9	15.3 (a)	.214	.219
60	20	20 (b)		

(a) = observed
(b) = calculated

due to its simplicity and controllability (31). It is generally believed that the interaction of alcohols and Ce^{4+} proceeds by formation of a coordination complex which then disproportionates to form a free radical (30); a mechanism is shown in Figure 57. The free radical then induces polymerization if any vinyl monomer is present.

The ceric ion method is usually used with homogeneous systems and heterogeneous systems where the substrate is swollen by solvent. In our study, the hydroxyl-bearing substrate is not swollen by solvent.

Figure 58 shows the FT-IR/ATR spectrum of the ceric ion graft polymerization of AM onto LDPE. An intense amide peak can be seen in the 1690-1550 cm^{-1} region. Another intense peak shows up at 1125 cm^{-1} corresponding to the ether group by which polyacrylamide is attached to the substrate. Exhaustive extraction in soxhlet with various solvents (CH_3OH, acetone, water) decreased only very slightly the amide peak.

The XPS analysis (Figure 59) shows 3 major peaks: carbon 1S at 285 ev, oxygen 1S at 531 ev, and nitrogen 1S at 401 ev. The quantitative XPS analysis (Table 9) confirms that a substantial grafting has occurred. If we make the rough assumption that the polyacrylamide chains are perpendicularly oriented to the surface and if we consider a thickness of 10 monolayers (~ 50Å) which is the average scanning depth of XPS, then it comes out that the theoretical and experimental atomic concentrations correlate well (Table 9). The discrepancy between the two results is within a reasonable range if we cnsider the effect of hydrocarbon contamination in the analysis chamber (41) and the operator-dependent experimental errors particularly from the quantification of the areas.

A comparison between plasma-induced and the cerium-induced graft polymerization of AM shows that the latter method offers a better yield. The main difference between the two systems is in the initiation processes.

5 CONCLUSIONS

In this study, we evaluated novel surface modifications of LDPE (low density polyethylene). Three methods relevant to the synthesis of highly hydrophilic surfaces for antibacterial activity and cell adhesion improvement were studied. The following conclusions can be drawn.

5.1 Chemical Modification

1. Chromic acid oxidation produced a highly polarized surface mainly containing carboxyl groups.
2. DCC was an effective dehydrating agent for the coupling of carboxyl and amine groups. The resulting covalent amide bond was strongly attached to the surface.
3. Alkylation of the terminal amine group attached to the surface through the amide bond was not possible. The inhibition of the reactivity of the amine could be mainly due to steric effect from the substrate.

5.2 Plasma-Induced Graft Polymerization

1. Acrylamide (AM) was successfully grafted onto LDPE, thus yielding a highly hydrophilic surface.

2. The ammonium salt of dimethylaminoethyl methacrylate (Q-DMAEMA) could not be grafted onto LDPE. Steric effect and aggregation of the monomer could be the limiting factors.
3. The graft polymerization of AM and Q-DMAEMA was successfully graft copolymerized onto LDPE.
4. The previous two conclusions suggest that plasma-induced polymerization is selective towards monomers -- this selectivity is not yet well understood.
5. Although the yields are still low, it was proved that the grafting of ammonium groups onto a highly hydrophobic surface is possible. Optimization of the experimental parameters should provide high grafting yields.

5.3 Ce^{4+}-Induced Graft Polymerization of AM onto LDPE

1. Diborane reduction of carboxylic groups was effective to generate hydroxyls on the surface of LDPE.
2. Ce^{4+} was proved to be a useful initiator system for the graft polymerization of AM onto LDPE. This could be extended to other vinyl monomers for specific surface modifications.
3. The FT-IR/ATR peak intensity of the acrylamide proved that a substantial grafting was obtained.

5.4 Characterization

1. XPS proved to be a valuable technique for elemental and chemical structure analyses. However, contamination due to a poor vacuum

6 FUTURE WORK

For future work we suggest the following guidelines for each modification procedure.

6.1 Chemical Modification

1. Chromic acid oxidation of linear low density polyethylene. This would provide a more homogeneous oxidized surface.
2. Use of longer spacer between the covalently attached amine group through an amide linkage. This would provide more mobility, a better solvation, thus a decreased steric effect and an enhanced reactivity of the amine.
3. Use of other alkylating agents such as dimethylsulfite which is more stable than alkyl iodides, therefore could withstand more severe reaction conditions.
4. Attachment of an alkyl halide to the LDPE surface and subsequent reaction with a tertiary amine could be a good alternative for synthesis of a quaternary ammonium group.

6.2 Plasma-Induced Polymerization

1. Improvement of the plasma reactor regarding the vacuum. A better vacuum (10^{-3}-10^{-4} torr) should provide more stability for the reactive species.

2. Use of other gases such as O_2 and water vapor to generate active species on the solid substrate.
3. Optimize the effect of operating parameters: power, time.
4. Investigate the use of other solvents on the effect of rate of polymerization.
5. Investigate other monomers and substrates.

6.3 Ce^{4+}-Induced Graft Polymerization of AM onto LDPE

1. Study the effect of initiator and monomer concentrations and compare with similar reactions in a homogeneous system.
2. Study the extent of homopolymer formation.
3. Study the effect of adding the reactants in different sequences such as
   (a) (1) substate + Ce^{4+}
       (2) add monomer
   (b) (1) substrate + monomer
       (2) add Ce^{4+}
   (c) (1) substrate + Ce^{4+}
       (2) remove excess Ce^{4+}
       (3) add monomer.

REFERENCES

1. Andrade, J. D., in Surface and Interfacial Aspects of Biomedical Polymers, Chap. 5, Andrade J. D., ed., Plenum Press, New York (1985).

2. Briggs D., Rance, D. G., Kendall, C. R., and Blythe, A. R., Polymer, 21, 895 (1980).

3. Dwight, D., Chemtech, March, 166 (1982).

4. Rasmussen, J. R., Stedronsky, E. R., and Whitesides, G. M., J. Amer. Chem. Soc., 99, 4736 (1977).

5. Rasmussen, J. R., Stedronsky, E. R., and Whitesides, G. M., J. Amer. Chem. Soc., 99, 4746 (1977).

6. Isquit, A. J., Abbott E. A., and Walters, P. A., Appl. Microbiol., 24, 859 (1972).

7. Nakagawa, Y., Yamano, Y., Towaratani, T., Kourai, H., Horie, T., and Shibasaki, I., Appl. Environ. Microbiol., 43, 1041 (1982).

8. Sharma, C. P., Biomaterials, 2, 57 (1981).

9. Hayward, J. A., and Chapman, D., Biomaterials, 5, 135 (1984).

10. Baier, R. E., Meyer, A. E., Natiella, J. R., Natiella, R. R., and Carter, J. M., J. Biomed. Mat. Res., 18, 337 (1984).

11. Marszalec, D. S. Gerchakov, S. M., and Udey, L. R., Appl. Environ. Microbiol., 38, 987 (1979).

12. Fletcher, M., and Pringle, J. H., J. Colloid Interface Sci., 104, 5 (1985).

13. Shilo, M., and Fattom, A., Appl. Environ. Microbiol., 47, 135 (1984).

14. Paul, J. H., and Jeffrey, W. H., Appl. Environ. Microbiol., 50, 431 (1985).

15. Baier, R. E., Shafrin, E. G., and Zisman, W. A., Science, 162, 1360 (1968).

16. Marshall, K. C., Stout, R., and Mitchell, R., Can. J. Microbiol., 17, 1413 (1971).

17. Marshall, K. C., Stout, R., and Mitchell, R., J. Gen. Microbiol., 68, 337 (1971).

18. Gatoire, B., Bouriot, P., Baszkin, A., Ter-Minassian-Saraga, L., and Boissonnade, M. M., J. Colloid Interface Sci., 79, 143 (1981).

19. Ericksson, J. C., Golander, C. G., Baszkin, A., and Ter-Minassian-Saraga, L., J. Colloid Interface Sci., 100, 381 (1984).

20. Nuzzo, R. G., and Smolinsky, G., Macromolecules, 17, 1013 (1984).

21. Smith, M., Moffatt, J. G., and Khorana, H. G., J. Amer. Chem. Soc., 80, 6204 (1958).

22. Andrew, W., and Ibrahim, I. T., Chem. Rev., 81, 589 (1981).

23. Hoover, M. F., and Butler, G. B., J. Polym. Sci., Symp. 45, 1 (1974).

24. Nakagawa, Y., Hayashi, H., Tawaratani, T., Kourai, H., Horie, T., and Shibasaki, I., Appl. Environ. Microbiol., 47, 513 (1984).

25. Yasuda, H., and Hsu, T., J. Polym. Sci., Polym. Chem. Ed., 15, 81 (1977).

26. Osada, Y., Bell, A. T., and Shen, M., J. Polym. Sci.: Polym. Lett. Ed., 16, 309 (1978).

27. Osada, Y., and Iriyama, Y., Thin Solid Films, 118, 197 (1984).

28. Johnson, D. R., Osada, Y., and Bell, A. T., Macromolecules, 14, 118 (1981).

29. Osada, Y., Takase, M., and Iriyama, Y., Polym. Journal, 15, 81 (1983).

30. Mino, G., and Kaizerman, S., J. Polym. Sci., 122, 242 (1958).

31. Vitta, S. B., Stahel, E. P., and Stannett, V. T., J. Macromol. Sci.-Chem., A22, 579 (1985).

32. Klausner, Y. S., and Bodansky, M., Synthesis, Sept., 453 (1972).

33. Sommer, H. Z., and Jackson, L. L., J. Org. Chem. 35, 1558 (1970).

34. Sommer, H. Z., Lipp, H. I., and Jackson, L. L., J. Org. Chem., 36, 824 (1971).

35. Everhart, D. S., and Reilley, C. N., Anal. Chem., 53, 665 (1981).

36. Blais, P., Carlsson, D. J., and Wiles, D. M., J. Appl. Polym. Sci., 15, 129 (1971).

37. Gerenser, L. J., Elman, J. F., Mason, M. G., and Pochan, J. M., Polymer, 26, 1162 (1985).

38. Briggs, D., Brewis, D. M., and Konieczo, M. B., J. Mat. Sci., 10, 1270 (1976).

39. Clark, D. T., and Wilson, R., J. Polym. Sci., Polym. Chem. Ed., 21, 837 (1983).

40. Strobel, M., Corn, S., Lyons, C. S., and Korba, G. A., J. Polym. Sci., Polym. Chem. Ed., 23, 1125 (1985).

41. Gilding, D. K., Paynter, R. W., and Castle, J. E., Biomaterials, 1, 163 (1980).

42. Larsson, N., Stenius, P., Ericksson, J. C., Maripuu, R., and Lindberg, B., J. Colloid Interface Sci., 90, 127 (1982).

43. Lavielle, L., and Schultz, J., J. Colloid Interface Sci., 106, 438 (1985).

44. Clark, D. T., and Feast, W. J., in Polymer Surfaces, pp. 213-234, John Wiley and Sons, New York, (1978).

45. Wu, S., in Polymer Interphase and Adhesion, Chapter 9, pp. 279-336, Marcel-Dekker, New York (1982).

46. Brewis, D. M., and Briggs, D., Polymer, 22, 7 (1981).

47. Gardella, J. A., Chen, J. S., Magill, J. H., and Hercules, D. M., J. Amer. Chem. Soc., 105, 4536 (1983).

48. Dias, A. J., and McCarthy, T. J., Macromolecules, 18, 1826 (1985).

49. Wiberg, K. B., and Eisenthal, R., Tetrahedron, 20, 1151 (1964).

50. Blais, P., Carlsson, D. J., Csullog, G. W., and Wiles, D. M., J. Colloid Interface Sci., 47, 636 (1974).

51. Briggs, D., in Practical Surface Analysis by Auger and X-Ray Photoelectron Spectroscopy, Chap. 9, pp. 359-396, Ed., Briggs, D., and Seah, M. P., John Wiley and Sons, Chichester (1983).

52. De Tar, D. F., and Silverstein, R., J. Amer. Chem. Soc., 88, 1013 (1966).

53. Odian, G., in Principles of Polymerization, Chap. 9, pp. 654-703, 2nd ed., Wiley-Interscience Publ., New York (1981).

54. Dias, A. J., and McCarthy, T. J., Macromolecules, 17, 2529 (1984).

55. Hoover, M. F., J. Macromol. Sci.-Chem., A4(6), 1327 (1970).

56. Yasuda, H., in Plasma Polymerization, Chap. 5, pp. 44-71, Academic Press Inc., Orlando (1985).

57. Nagai, K., Ohishi, Y., Inaba, H., and Kudo, S., J. Polym. Sci., Polym. Chem. Ed., 23, 1221 (1985).

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Ali Yahiaoui was born on March 21, 1952 in Azeffoun, Algeria. He attended high school in Algiers and received a "Baccaulerat-Sciences Experimentales" in June 1973. In September of that year he entered the Department of Chemistry of the "Faculté des Sciences" of the University of Algiers. In June 1977 he received his "Diplome d'Etudes Supérieures" in applied organic chemistry. From May 1978 to May 1980 he attended the army to satisfy his military obligations. He started his civilian job career in June 1980 as a process engineer in the Research and Development Department of the Algerian Oil Company, Sonatrach. He came to the USA in January 1982 where he first attended the Intensive English Program of the University of Texas at Austin, Texas, to learn English. In the spring of 1983 he entered graduate studies in the Department of Chemistry, University of Florida. In the fall of 1984 he moved to the Department of Materials Science and Engineering, at the same university. While pursuing a master's degree at the University of Florida, the author has served as a graduate research assistant and was a member of the American Chemical Society.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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